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THE INTERRELATION OF THE SURVIVING HEART AND PANCREAS OF THE DOG IN SUGAR METABOLISM.

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The problem of sugar metabolism and diabetes is intimately associated with the two following questions: (1) Does the pancreas have a local independent action on dextrose passing through its blood vessels, altering the dextrose in some way for utilization by the body tissues? (2) Is there a direct interrelation between the pancreas and body tissues in dextrose metabolism, and if so, how is it accomplished?

In the experimental work reported below the attempt has been made to gain some evidence as to the first of these questions by the direct perfusion of the pancreas with physiological concentrations of dextrose. The second question has been studied by various types of perfusion experiments on the isolated surviving heart and pancreas in order to determine whether there was any interdependence between the two in dextrose metabolism.

The numerous experiments recorded by various workers bearing upon the interrelation between the pancreas and body tissues in the utilization of dextrose need not be reviewed in detail. It is sufficient to say that two general lines of investigation have developed. On the one hand, the experiments of Hédon (1), Carlson and Ginsburg (2), Forschbach (3), Carlson and Drennan (4), Verzár and von Fejér (5), Woodyatt (6), Murlin and Kramer (7), and others, in which blood was transferred in various ways from normal to diabetic animals in the hope of thus supplying a pancreatic hormone, have yielded no decisive results. Again, Verzár and von Fejér (5), Murlin and Kramer (7), Scott (8), and others have attempted to supply this hypothetical internal secretion by the injection into diabetic animals of various kinds of pancreatic extracts. Their results have in general been negative. The obvious objection can be raised to all such experiments that in grinding the pancreas its powerful proteolytic enzymes might readily inactivate a more delicate product of internal secretion.

TABLE I.

Summary of Experiments on the Utilization of Dextrose by the Isolated Mammalian Heart.

Author	Date.	Type of heart.	No. of experiments.	Circulating medium.	Strength of dextrose perfused.	No. of hrs. perfused.	Interval between sugar determinations.	Dextrose disappeared per gm. of heart per hr.	Bacterial counts.	Final perfusate hydrolyzed.	Aseptic precautions. Special notes.
					per cent			*mg.*			
J. Müller (17).	1904	Cat.	4	Ringer-Locke's.	0.1 ±	2–6	At end of experiment.	2 ±	None.	No.	Not mentioned. Heart estimated as 12 gm.
Locke and Rosenheim (18)	1907	Rabbit.	4 beating. 3 not beating.	"	0.1–0.25	7–10	At beginning and end of experiment.	11.2–1.7	"	Yes.	Not mentioned.
Camis (19)....	1908	"	20	"	0.1 ±	25–69 min.	At end of experiment.	0	"	No.	Not mentioned. Cat's hearts estimated as 12 gm. Rabbit's hearts estimated as 8 gm.
		Cat.	9					13			
Rohde (20)...	1910	"	12	"	0.2	4 ±	At 2nd and 4th hrs.	0.98 1st. 1.64 2nd.	"	"	Apparatus washed with alcohol. Aseptic surgical technique not mentioned.
Gayda (21)...	1912	Rabbit.	10	"	0.1	4–6	At end of experiment.	3.8–14.9 Av. 7.1	"	"	Apparatus washed with alcohol. Aseptic surgical technique not mentioned.
Knowlton and Starling (22)	1912	Dog.	10 normal. 7 diabetic.	Heart-lung preparation.	0.2–0.89	1–3	1 hr. ±	Normal 4 Diabetic 0	"	"	Attempt at sterilization of apparatus in a few experiments. Aseptic surgical technique not mentioned.
Neukirch and Rona (23)...	1912	Cat.	Glucose 1.	Ringer-Locke's.	1.0	6½	At end of experiment.	1.6	"	"	Apparatus sterilized with concentrated acid and water. Aseptic surgical technique not mentioned.

Maclean and Smedley (24)	1913	Rabbit. Dog.	4 3	Ringer-Locke's.	0.1–0.2	1–4	Successive hrs.	0.7 (av.) 1.3 (av.)	None.	No.	Apparatus sterilized in autoclave. "Ordinary aseptic precautions."
Maclean and Smedley (25)	1913	Diabetic dog.	12	"	0.1–0.3	3		0 +	"	"	Apparatus sterilized in autoclave. "Ordinary aseptic precautions."
Mansfeld (26).	1913	Rabbit.	5 normal. 8 fever.	"	0.1	3	At 1½ and 3 hrs.	Normal 2.2 Fever 4.4	"	"	Not mentioned.
Patterson and Starling (27)	1913	Dog.	9 normal. 14 diabetic.	Heart-lung preparation.	0.2–0.7	1–3 2	At end of experiment.	Normal 0+ Diabetic 0+	"	"	Apparatus washed with soda and occasionally boiled. Aseptic surgical technique not mentioned.
Cruickshank (28).....	1913	"	6 normal. 13 diabetic.	Heart-lung preparation.	0.4–0.6	1–4 1½–4			"		Same as preceding experiment. Experiments conducted to study glycogen content of hearts.
Cruickshank and Patterson (29)....	1913	Cat. Dog.	14 8 diabetic.	Ringer-Locke's.	0.2–0.3	1–3	Perfusions divided into two periods.	1.5 0.9	"	Yes.	Apparatus washed with boiling water. Aseptic surgical technique not mentioned.
Starling and Evans (30)..	1914	"	Numerous.	Heart-lung preparation.	?	Varying.		Estimated from RQ. 1.6	"	No.	Not mentioned.
Underhill and Prince (31)..	1914	Rabbit.	25	Ringer-Locke's.	0.1	2	At end of experiment.	0.7–3.8	"	"	Apparatus sterilized in autoclave. State that aseptic precautions were observed.

The second main line of investigation has developed since Cohnheim (9) in 1903 reported experiments in which he found that while pancreatic extract or muscle extract alone had no effect on dextrose, a mixture of the two caused a rapid disappearance of sugar, which he interpreted as being due to an oxidative process. Although his results were attacked by Claus and Embden (10) and others, they were supported by Arnheim and Rosenbaum (11), Dewitt (12), and Hall (13), and were generally accepted until Levene and Meyer (14) in 1911 found that this apparent destruction of dextrose was due to a condensation process, for by hydrolysis the reducing sugar was restored.

Method.

The method of isolated perfusion was chosen in the following experiments, for, while obviously artificial compared to normal physiological conditions, it offered a method of controlling the factors concerned.

Two references only to the direct perfusion of the pancreas have been found. Hustin (15) perfused the pancreas in a study of its external secretion, and de Meyer (16) in 1910 found that by adding a solution previously perfused through a pancreas to one subsequently perfused through a liver there was an increase in the liver glycogen. The findings of fifteen observers who have studied the utilization of sugar by the isolated mammalian heart are summarized in Table I. An analysis of their results brings out the following criticisms.

1. A uniform type of animal has not been used.

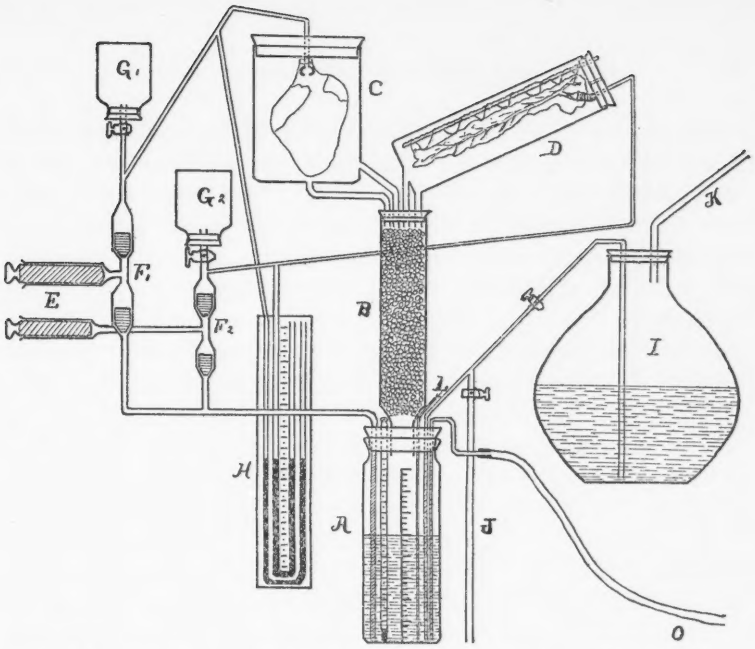
2. The conclusions of the different observers are based on no constant time period of perfusion, but vary from 25 minutes to 10 hours.

3. Save for the experiments of Maclean and Smedley (24), there has been no attempt to make hourly sugar determinations in order to follow the rate of sugar disappearance.

4. There has been a great discrepancy between the findings of various workers as to the amount of dextrose used by the living heart. Patterson and Starling (27) in the most recent work using a heart-lung preparation and correcting for the sugar destroyed in the lung found very little if any sugar used either by a normal or diabetic heart in periods of 1 to 3 hours.

5. Locke and Rosenheim (18) and Cruickshank and Patterson (29) are the only workers who hydrolyzed the final perfusates to determine whether by so doing there was an increase in the reducing substance present.

6. The observance of aseptic precautions has varied greatly with different workers. Maclean and Smedley (25) and Underhill and Prince (31) alone state that their apparatus was sterilized in an autoclave. In no instance save in the experiments of Underhill and Prince is there mention of scrupulous, aseptic, surgical technique, and in no instance are bacterial counts given.



TEXT-FIG. 1. Diagram of the perfusion apparatus. Reduced to about one-sixth.

A. Reservoir containing the perfusion fluid, consisting of a 400 cc. graduated glass bottle with a rubber stopper through which the various tubes and thermometer pass.

B. Artificial lung consisting of a glass cylinder filled with glass beads.

C. Glass perfusion chamber in which the heart is suspended from the bulb-shaped cannula tied in the aorta. The cannula passes through the large rubber cork. The fluid after perfusing through the coronary arteries flows from the auricles and passes down through the lung into the reservoir.

D. Glass perfusion chamber in which the pancreas is suspended by its mesenteric border to the long glass arm which projects from the rubber cork. The cork is perforated by an opening for the escape of oxygen and one for transmitting the cannula to the pancreatic artery. The perfused fluid drips from the pancreas and flows down through the lung to the reservoir.

E. 3 and 5 cc. Luer syringes acting as pumps, and operated by a motor attachment.

F_1 and F_2. Glass two-way valves.

G_1 and G_2. Glass bottles acting as air-heads.

H. Double manometer connected with each of the delivery systems.

I. Reserve reservoir with syphon connection to the perfusion reservoir.

J. Tube for draining the perfusion reservoir.

K. Blow-tube for starting the syphon.

L. Small tube for removing samples of perfusate for analysis.

O. Oxygen tube. The oxygen bubbles through the reservoir and passes up through the column of beads against the stream of perfusate coming down from the organs.

The importance of this last criticism has been emphasized by the results of this investigation. Infection of the perfusing liquid occurs easily and its occurrence may account for a slight or even a considerable disappearance of dextrose. Extreme care in sterilization of the apparatus and the observance of strict aseptic precautions are necessary to exclude this factor. All my final experiments were controlled by bacterial counts, and those in which the precautions against infection were not successful, as shown by the bacterial counts, were discarded. It seems evident that previous workers have failed to realize that not only must ordinary aseptic precautions be used, but that only by the minute observance of the most rigid care controlled by bacterial counts can bacteria be sufficiently excluded to make the conclusions as to sugar utilization in such experiments reliable.

Apparatus.—The diagram of the apparatus used is shown and explained in Text-fig. 1. Extreme care was taken to wash all adherent fat and protein from the apparatus immediately at the end of each experiment. Sterilization was accomplished by fractional sterilization in an Arnold sterilizer. Immediately before each experiment the apparatus was tested by perfusing several times with sterile Locke's solution containing the percentage of dextrose to be used in the experiment. This technique was time-consuming but was justified by repeatedly getting an apparatus, washings from which showed no bacteria on agar plates. The temperature of the apparatus and perfusate in all experiments was maintained at 37–38.5°C. by keeping the apparatus in a thermostat.

Perfusate.—The solution used in all experiments was a standard Ringer-Locke's solution of the following formula: calcium chloride 0.024 per cent, potassium chloride 0.042 per cent, sodium bicarbonate 0.03 per cent, sodium chloride 0.9 per cent dextrose (Kahlbaum's pure) 0–0.5 per cent (according to the experiment). Locke's solution was made up immediately before each experiment from freshly distilled water which was sterilized by boiling. The amount of perfusate was generally 250 cc. and was left unchanged during the period of perfusion, save for samples removed for analysis.

Dextrose Determinations.—A slight modification of the colorimetric method of blood sugar determination described by Lewis and Benedict (32) was used. The great advantage which the method offered was

that the small amount of fluid necessary for analysis did not materially diminish the volume of the perfusate, so that hourly observations were possible. The results reported in these experiments obviously depend on the accuracy of the technique of sugar determination. For this reason in the earlier experiments hourly duplicate determinations were made not only on the perfusate but on the control perfusion and on the original Locke's solution. The average error of duplicate determinations practically never exceeded 0.5 per cent of the total sugar present. Correction was always made for the amount of perfusate removed for analysis in estimating the total volume of sugar present in the perfusate.

Controls.—A small apparatus similar in all respects to the main apparatus save that it contained no living tissue was used in the first forty-seven experiments. Its reservoir of 50 cc. was filled from the main apparatus after the heart or pancreas or both had been perfused for 10 minutes. Thus the control apparatus contained a fluid which was inoculated with whatever bacteria were contained in the main perfusate and with whatever enzymes were washed out in that time. In no experiment considered was there any change in the reducing properties of the sugar in the control perfusion beyond the limits of experimental error.

Bacterial Counts.—The plate method of bacterial counting was used throughout. Contaminating organisms were usually of the intestinal types. While contamination generally occurred even with the most rigid precautions, it was possible to estimate from the number of bacteria present whether they were sufficient to account for a disappearance of sugar. Six experiments were obtained which remained entirely sterile for the 4 hour period of perfusion. Those experiments were discarded in which bacterial contamination might in any way be interpreted as having accounted for dextrose utilization.

Hydrolysis.—The method of hydrolysis used by Levene and Meyer (14) was followed: hydrolyzing for 2 hours with 1 per cent hydrochloric acid with a reflux condenser, and subsequently carefully neutralizing and bringing to the original volume. It was found that this percentage of acid gave the maximum hydrolytic effect. If a polymerization of the dextrose into some more complex, non-reducing form occurred, hydrolysis should restore it to the simple mono-

saccharide. In this way it is possible to determine whether the disappearance of dextrose is a real utilization or only an apparent one.

Surgical Procedure.—Small or medium sized dogs were used which had been previously fed on a routine meat-bread diet. Great care was observed in shaving and sterilizing the chest and abdomen of each dog. All instruments were sterilized by boiling. Rubber gloves and sterile gowns were worn by the operator and his assistant. The table and animal, except the narrow field of operation, were covered with sterile towels. In a word, every precaution of a major surgical operation was used. The dogs were anesthetized with ether.

Removal of the Pancreas.—The removal and perfusion of the pancreas is a simple operation. The abdomen was opened by a transverse incision just below the costal margin. The aorta was clamped as it emerged from the diaphragm thus making the subsequent steps practically bloodless. The pancreaticoduodenal artery was exposed and a cannula placed in it. All the pancreas except the splenic portion which is not supplied by the pancreaticoduodenal artery was then gently removed. The common bile-duct must not be cut. The pancreas was then washed in warm sterile Locke's solution and loosely suspended by a continuous suture through its mesenteric border to the supporting arm of the perfusion chamber and placed in the apparatus, as shown in Text-fig. 1. The cannula was connected and the perfusion started. The procedure can be carried out in less than 5 minutes. A pulsatile pressure whose systolic pressure never exceeded 40 mm. of mercury was used, and about 20 cc. of fluid were perfused per minute. Carmine gelatin injections of the pancreas by this method showed that there was complete penetration of the fluid to every portion of the pancreas.

Removal of the Heart.—The technique of removal and perfusion of the heart scarcely need be described as it was similar to that used by previous workers. The thorax was rapidly opened, the heart removed and transferred to a dish of warm sterile Locke's solution, where, after washing it free of any excess of blood, the cannula was tied into the aorta above the aortic valves. Care must be used to exclude air bubbles. The heart was placed in the perfusion chamber and perfusion begun at once. This procedure requires about 3 min-

utes. The heart generally beats vigorously from the first. At the end of 4 hours the heart always showed a varying amount of edema and dilatation. As soon as the hearts were allowed to die, however, and rigor mortis set in, a considerable amount of this fluid seemed to be squeezed out. In addition every heart was mechanically squeezed free of as much fluid as possible. From gross appearance the heart approximated its original size. The error that resulted from this procedure must have been slight, and fairly uniform for all the experiments. In the last thirteen experiments the hearts were weighed before and after perfusion and the results justified the previous method.

The amount of fluid perfused through the hearts varied from 20 to 60 cc. per minute according to the size of the heart. To force this amount of fluid through the coronary arteries required a pulsating pressure whose systolic pressure varied from 40 to 160 mm. of mercury, and the diastolic pressure from 20 to 100 mm. What factor caused this variation in the resistance of the coronary arteries has not been determined. In all experiments recorded, unless otherwise noted, the hearts continued to beat for the 4 hour period.

Perfusion of the Heart and Pancreas.—In a simultaneous perfusion of the heart and pancreas from the same animal the heart was first removed and then the pancreas, as described above. The double perfusion is shown in Text-fig. 1. It is obvious that the intimate mixture of the fluids passing through the two organs was practically equivalent to a perfusion of the organs in series. If the pancreas was to be perfused first it was removed and the dog was kept under light ether anesthesia until the heart was required.

RESULTS.

Pancreas Perfusions.—Eleven experiments were carried out before the technique of perfusion was perfected. In these experiments there was a distinct disappearance of dextrose from solutions perfused through the pancreas. This might have been interpreted as a utilization of, or change in the dextrose, had bacterial counts not been made. Table II is given to illustrate these experiments, and shows that at the end of 5 minutes the perfusate contained about 350,000 bacteria per cc. In the first 3 hours there was a slight drop in the

TABLE II.

Infected Pancreatic Perfusion.

Fluid.	Sugar, 5 min.	Sugar, 3 hrs.	Sugar, 6 hrs.	Bacteria per cc. at end of 5 min.	Bacteria per cc. at end of 6 hrs.
	per cent	*per cent*	*per cent*		
Original Locke's solution.....	0.3041	0.3030	0.3035	0	0
Control perfusion.....	0.3041	0.3058	0.3041	350,000	19,400,000
Pancreatic perfusion.....	0.3026	0.300	0.2347	350,000	50,500,000

percentage of dextrose. In the subsequent 3 hours there was a considerable loss of sugar but the bacterial counts at the end of this 6 hour perfusion showed over 50,000,000 bacteria per cc. With perfection of the technique, however, three successive perfusions of the pancreas were obtained in which the maximal bacterial count was 1,670,000. In none of these experiments was there the least detectable loss of dextrose. Table III shows the summarized results of these experiments. In these experiments it seems evident that when

TABLE III.

Pancreas Perfusion.

Experiment No.	5 min.		1 hr.		2 hrs.		3 hrs.	
	Dextrose in perfusate.	Dextrose in control.	Dextrose in perfusate.	Dextrose in control.	Dextrose in perfusate.	Dextrose in control.	Dextrose in perfusate.	Dextrose in control.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
22.....	0.298	0.298	0.300	0.299	0.298	0.299	0.300	0.300
23.....	0.601	0.601	0.599	0.597	0.601	0.600	0.599	0.601
24.....	0.10	0.10	0.099	0.099	0.100	0.099	0.10	0.10

Experiment No.	4 hrs.		5 hrs.		6 hrs.		Bacteria.		Dextrose after hydrolysis.	Dextrose disappeared per gm. of pancreas.
	Dextrose in perfusate.	Dextrose in control.	Dextrose in perfusate.	Dextrose in control.	Dextrose in perfusate.	Dextrose in control.	5 min.	6 hrs.		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*			*per cent*	
22.....	0.298	0.299	0.298	0.299	0.300	0.300	40	22,560	0.296	0
23.....	0.600	0.600	0.600	0.600	0.601	0.599	12	295,000	0.599	0
24.....	0.099	0.10	0.099	0.099	0.099	0.10	101	1,670,000	0.099	0

dextrose is perfused through the pancreas there is neither a change of the dextrose into a non-reducing form, nor does hydrolysis alter its reducing properties.

Heart Perfusions.—In view of the variations reported by different workers as to the amount of dextrose utilized by the surviving heart, and in view of the obvious criticisms stated above, it was felt that before proceeding to perfuse the heart and pancreas together it would be necessary to determine the utilization of dextrose by the heart alone under the conditions used in these experiments. Five successful perfusions of the heart were obtained over a period of 4 hours in which the maximal bacterial count was 216,000 per cc. Table IV is a condensed chart of these experiments, and they are shown in an average curve in Text-fig. 2. The experiments indicate that during the 1st hour, in averaging the five experiments, the sugar utilization was 0.028 mg. per gm. of heart, in the 2nd hour 0.013 mg., in the 3rd hour 0.242 mg., and in the 4th 0.415 mg. It was felt that this disappearance of sugar in the last 2 hours was not due to bacterial action, for not only was there no loss of dextrose from the control perfusions, but in the perfusions of the pancreas for periods of 6 hours in which the maximal bacterial count exceeded any of those in the heart perfusions there was no loss of dextrose. In these experiments when the final perfusate was hydrolyzed there was no definite increase in reducing substance.

Heart-Pancreas Perfusions.—Six experiments were obtained in which the heart and pancreas were simultaneously perfused with the same fluid and in which the maximal bacterial count at the end of 4 hours was about 800,000 per cc. In two of these experiments, as will be seen by consulting Table V, the perfusing liquid remained practically sterile throughout. In Experiment 32 the heart suddenly stopped at the end of about $2\frac{1}{2}$ hours. The figures, however, are given. The results are diagrammatically shown in Text-fig. 2 and summarized in Table VI.

As the experiments progressed it became apparent, as shown in Text-fig. 2, that a striking phenomenon was occurring. The disappearance of sugar began in the 1st hour and was greatly in excess of that occurring when the heart was perfused alone. In the heart perfusions the distinct disappearance of sugar begins late, after the

TABLE IV.

Condensed Table of Experiments in Which the Heart Was Perfused Alone.

Experiment No.	Weight of heart.	Dextrose after 10 min. perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.	Average error of duplicate determinations.	Dextrose disappeared from control perfusion.
			Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.			
	gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*	*per cent*	
26.....	84	0.200	0.201	0	85	0.199	0.03	83	0.200	0	1,888	0.192	0.237	3,500		0.66	0
27.....	65	0.300	0.299	0.011	608	0.299	0	9,920	0.299	0		0.273	0.91			0.25	0
28.....	46	0.368	0.368	0.018	190	0.367	0.036	258	0.366	0.043	53,200	0.3655	0.025	216,000	0	0.12	0
29.....	69	0.501	0.497	0.11	44	0.499	0	7,100	0.473	0.933	7,800	0.456	0.678		0	0.65	0
34.....	82	0.099	0.100	0	97	0.099	0	288	0.091	0.237	7,600	0.083	0.226	57,200	0	0.2	0

TABLE V.

Condensed Table of Experiments in Which the Heart and Pancreas Were Perfused Together Simultaneously.

Experiment No.	Weight of heart.	Dextrose after 10 min. perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.	Average error of duplicate determinations.	Dextrose disappeared from control perfusion.
			Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.			
	gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*	*per cent*	
30.....	97	0.196	0.179	0.465		0.167	0.306	12,200	0.143	0.60	23,600	0.126	0.42	50,400	0.49	0.49	0
31.....	128	0.399	0.333	1.28	50,000	0.286	0.92	200,000	0.273	0.212	500,000	0.272	0.042	800,000	0.27	0.5	0
32.....	128	0.300	0.272	0.531	49,600	0.231	0.756	512,000	2½ hrs. 0.213	0.34					0.031	0.4	0
33.....	102	0.100	0.077	0.557	960	0.058	0.45	16,700	0.041	0.395	108,460	0.025	0.384	356,000	0.19	0.32	0
37.....	86	0.100	0.089	0.30	1	0.054	0.964	1	0.035	0.532	2	0.026	0.245	2	0.54	0.32	0
39.....	98	0.200	0.165	0.872	1	0.142	0.57	0	0.128	0.356	4	0.123	0.113	26	0.47	0.34	0

TABLE VI.

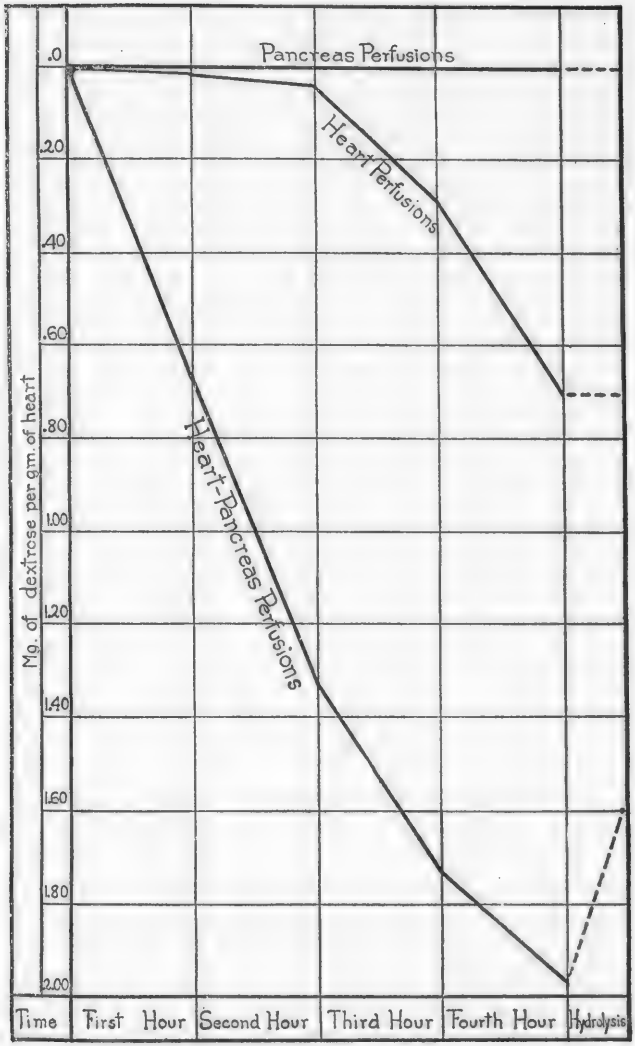
Table Combining the Results Given in Tables III, IV, and V.

	1st hr.	2nd hr	3rd hr.	4th hr.	Recovered by hydrolysis.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Average utilization of dextrose per gm. of pancreas in 3 perfusions.	0	0	0	0	0
Average utilization of dextrose per gm. of heart in 5 perfusions....	0.028	0.013	0.242	0.415	0
Average utilization of dextrose per gm. of heart in 6 heart-pancreas perfusions.....	0.667	0.661	0.406	0.200	0.332

2nd hour of perfusion, but from this point on, during the period of observation, it occurs in an increasing ratio. When the heart and pancreas are perfused together a striking loss of sugar occurs in the 1st hour, and towards the latter half of the experimental period of 4 hours this disappearance of sugar shows a decreasing ratio. Text-fig. 2 illustrates this point. The further fact also became apparent that hydrolysis of the final perfusates invariably gave a moderate but definite increase in the reducing substance. After deducting this amount of reducing substance recovered by hydrolysis, the amount of dextrose utilized by the heart in these experiments exceeded considerably that utilized by the heart alone. This is indicated in the tables and figures. In no instance did the control perfusion show any variation in reducing sugar beyond the limits of experimental error. From these experiments, then, it seemed that the pancreas not only enabled the heart in some direct way to utilize dextrose, but some alteration in the dextrose also occurred whereby it was changed in part at least to a non-reducing form which could be transformed back to dextrose again by hydrolysis.

Was This Utilization of Dextrose a Tissue Extract Phenomenon?

Was the disappearance of dextrose when the heart and pancreas were perfused together an effect similar to that obtained when muscle and pancreas extracts are allowed to act on dextrose? A number of heart-pancreas perfusions, which were considered as failures because



TEXT-FIG. 2. Curves showing the utilization of dextrose when the pancreas is perfused alone, when the heart is perfused alone, and when the two are perfused simultaneously. Note the increase in reducing substance in the heart-pancreas perfusions on hydrolysis.

TABLE VII.

The Effect of Pancreatic Perfusates and Pancreatic Extracts on Non-Beating Hearts.

Experiment No.	Procedure of experiment.	Weight of heart.	Dextrose after 10 min. perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.		
				Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.
		gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*	
36	Non-beating heart-pancreas perfusion.....	80	0.100	0.100	0	4	0.099	0	0	0.099	0	0	0.100	0	0
43	Non-beating heart perfused with pancreas extract.....	106	0.200										0.200	0	0
45	Non-beating heart perfused with pancreas extract.....	94	0.146										0.145	0.002 per hr.	1,260
62	Heart and pancreas frozen then perfused.	165	0.199	0.200	0		0.200	0		0.200	0		0.200	0	

the heart stopped beating soon after the perfusion was begun, showed practically no utilization of dextrose. The results of four such experiments are given in Table VII. In Experiment 36 the heart stopped beating almost immediately at the beginning of the perfusion. In Experiment 62 the heart and pancreas were frozen on removal from the body and then perfused. In Experiments 43 and 45 the pancreas was removed and ground, with all aseptic precautions, extracted with Locke's solution, and filtered. The hearts were then perfused with this extract. The hearts stopped beating almost instantly and the perfusion pressures rose to over 250 mm. of mercury. The perfusions were continued above this pressure, however. In none of these four experiments was there any disappearance of dextrose over a 4 hour period.

Again, in all the heart-pancreas perfusions reported above, and others to be mentioned below, the final perfusates were placed in the thermostat with chloroform and toluene as preservatives. If the dextrose utilization had been due merely to the extracts of the heart and pancreas the disappearance of dextrose ought to continue in the fluid after perfusion. It was invariably found, however, that not only was there no subsequent disappearance of dextrose in these perfusates, but that they generally showed an increase in the amount of reducing substance present. The immediate cessation of glycolytic action was probably not due to the preservatives used, for the experiments of most of the workers from Cohnheim to Levene and Meyer have been based on the effect of tissue extracts preserved with similar antiseptics. It is also to be emphasized that if the disappearance of dextrose was due to the tissue extracts, some change might be expected in the control perfusions which had passed for 10 minutes through the vessels of the two organs. The control perfusions, however, did not show any change, and this fact is discussed further below. Finally, in the experiments of Levene and Meyer (14) this phenomenon occurred only with strengths of dextrose greatly in excess of the physiological amounts used in these experiments, and on hydrolysis they were able to obtain practically all the original sugar. In my experiments only a small portion of the reducing substance could be recovered by hydrolysis. It is believed, therefore, that the results of these experiments indicate that the utilization of dextrose in the

heart-pancreas perfusions in which the heart was actively beating was not a phenomenon similar to that obtained when muscle and pancreas extracts are allowed to act on more concentrated solutions of dextrose, but that in these experiments the utilization of dextrose was dependent on the activity of the living heart tissues.

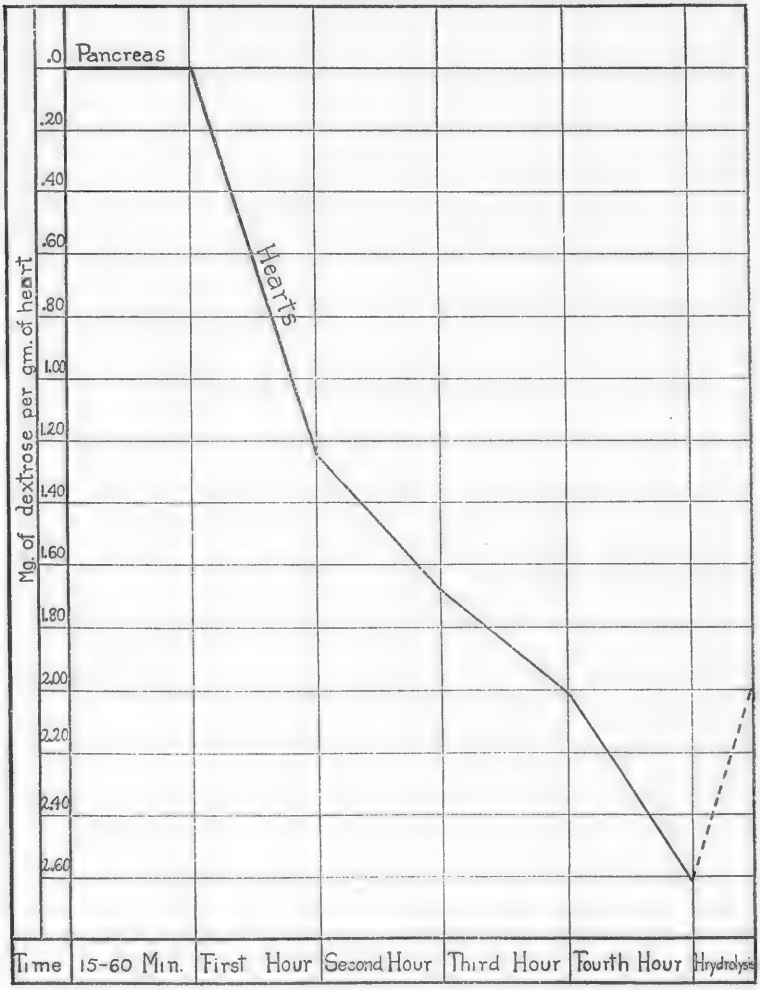
The Pancreas First Perfused and Subsequently the Heart Perfused with the Same Solution.

The question next arose as to whether the pancreas supplied anything to the perfusing Locke's solution which could subsequently cause the utilization of dextrose by the heart. Six experiments were conducted to determine this point. The results are given in Table VIII, and charted in Text-fig. 3. In Experiments 40 and 42 the pancreas was first perfused for 1 hour with Locke's solution containing dextrose. The dog was meanwhile kept alive under light ether anesthesia. The pancreas was then removed from the apparatus, and the heart substituted and perfused with the same solution that had been perfusing through the pancreas. The utilization of dextrose by the heart proceeded exactly as if the pancreas were in circuit, and hydrolysis of the final perfusate gave an increase in the reducing substance. Four experiments were then carried out in which the pancreas was first perfused for a period of 15 to 40 minutes with a Locke's solution containing no dextrose. The pancreas was then removed from the apparatus and sufficient dextrose added to the perfusing solution to give approximately the desired per cent. After allowing it to mix thoroughly in the apparatus, perfusion of the heart was begun. Again all the experiments showed rapid dextrose utilization and, as in the heart-pancreas perfusions, hydrolysis of the final perfusates increased their reducing properties. Two facts thus seemed evident. First, the pancreas did not alter the reducing properties of the dextrose passing through its vessels. Second, the pancreatic factor causing the rapid disappearance of sugar was contained in the perfusate and continued to act after removal of the pancreas. The pancreas, therefore, had furnished some constituent to the perfusing liquid which greatly accelerated the utilization of sugar by the heart muscle.

TABLE VIII.

Experiments in Which the Heart Was Perfused with a Solution Which Had Previously Been Perfused through the Pancreas. In the Last Four Experiments the Pancreas Was Perfused with Locke's Solution Containing No Dextrose. After Removal of the Pancreas Sufficient Dextrose Was Added to the Perfusate to Give the Desired Per Cent.

Experiment No.	Length of time pancreas perfused.	Dextrose utilized by pancreas.	Weight of heart.	Dextrose after 10 min. heart perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.
					Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	
			gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*
40	1 hr.	0	82	0.100	0.091	0.277	12	0.077	0.41	220	0.064	0.38	164	0.048	0.600	64	0.422
42	1 "	0	81	0.199	0.167	0.99	5	0.153	0.442	36	0.153	0	121	0.123	0.87	3	0.5
44	40 min.	0	119	0.297	0.160	2.76	0	0.133	0.555	0	0.109	0.40	1	0.092	0.28	5	0.43
46	15 "	0	91	0.246	0.176	2.1	0	0.170	0.158	0	0.121	1.22	0	0.109	0.28	0	0.94
47	40 "	0	105	0.096	0.072	0.63	0	0.059	0.386	0	0.051	0.17	0	0.051	0	0	0.465
48	40 "	0	105	0.30	0.272	0.761	0	0.24	0.605	0	0.217	0.422	0	0.171	0.86	0	0.55



TEXT-FIG. 3. The average curve of the six experiments in Table VIII. The pancreas was first perfused for periods varying from 15 to 60 minutes, and then removed from the apparatus and the heart of the same dog substituted. There was no dextrose utilization by the pancreas, but when the heart was subsequently perfused with the fluid which had been perfused through the pancreas, the heart-pancreas type of dextrose utilization occurred.

Effect of Heat on Pancreatic Perfusates.

If this pancreatic factor accelerating dextrose utilization by the heart is an enzyme, it should be affected by heat; while if it has a more stable character like adrenalin it should not be affected by heat to any extent. The five experiments on this point are tabulated in Table IX and shown in Text-fig. 4. In all the experiments the pancreas was perfused for 30 minutes and the perfusate was then removed from the apparatus. After completion of the pancreas perfusion the apparatus was carefully washed and sterilized in the steam sterilizer for 30 minutes. The heart was then perfused with the pancreatic perfusate which meanwhile had been subjected to various degrees of heating. In Experiments 51 and 52 the pancreatic perfusate was just brought to the boiling point and cooled, while in Experiment 55 the perfusate was boiled for 10 minutes. In Experiments 53 and 54 the pancreatic perfusates were heated to 56°C. for 30 minutes on a water bath, then cooled and perfused through the heart. In all these experiments the heart continued to beat during the 4 hour period. The results were definite, as shown in Text-fig. 4. When the pancreatic perfusate was heated to boiling it lost its power of enabling the heart to utilize dextrose. The utilization of dextrose proceeded practically as if the heart were being perfused alone. Heating the pancreatic perfusate to 56°C. for 30 minutes, however, did not cause any inactivation. Subsequent perfusion through the heart gave the typical heart-pancreas type of dextrose utilization. These experiments, therefore, indicated that the pancreatic factor has the characteristic of an enzyme in being inactivated by boiling temperature.

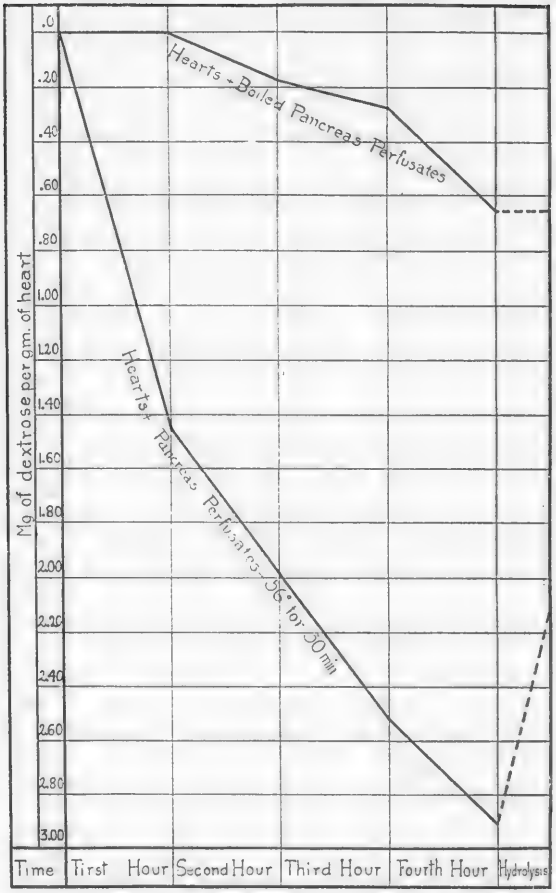
Amount of Pancreatic Perfusate Necessary to Enable the Heart to Utilize Dextrose.

In the experiments shown in Table VIII in which the pancreas was first perfused and the same solution was subsequently perfused through the heart, the entire pancreatic perfusate of 250 cc. was used. The question arose as to the minimum amount of pancreatic perfusate necessary to enable the heart to utilize dextrose immediately. Four experiments were carried out as shown in Table X and

TABLE IX.

Condensed Table of Experiments Showing the Effect of Heat on the Pancreatic Perfusate.

Experiment No.	Pancreas perfused 30 min. Perfusate removed and apparatus sterilized. Heart then perfused with pancreatic perfusate treated as follows:	Dextrose utilized by pancreas.	Weight of heart.	Dextrose after 10 min. heart perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.
					Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	
			gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*
51	Pancreas perfusate brought to boiling temperature	0	50	0.119	0.119	0	0	0.108	0.523	0	0.093	0.139	9	0.085	0.204	4	0.03
52	Pancreas perfusate brought to boiling temperature	0	63	0.120	0.120	0	0	0.120	0	0	0.120	0	0	0.099	0.711	9	0
55	Pancreas perfusate boiled for 10 min	0	62	0.200	0.199	0	5	0.200	0	6	0.196	0.14		0.188	0.254	34	0
53	Pancreas perfusate heated to 56° C. for 30 min	0	72	0.200	0.154	1.55	1	0.137	0.563	0	0.126	0.337	4	0.113	0.496	17	0.71
54	Pancreas perfusate heated to 56° C. for 30 min	0	60	0.200	0.166	1.35	1	0.153	0.496	2	0.133	0.733	5	0.125	0.286	3	0.86

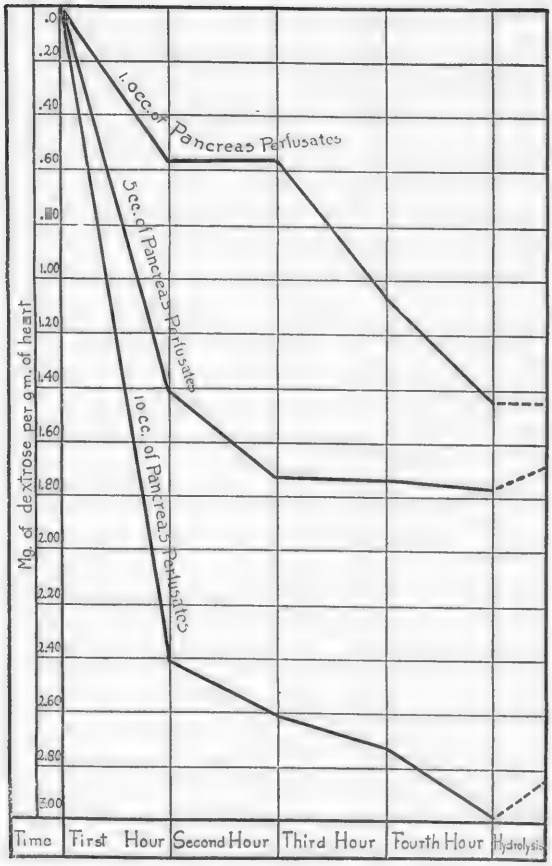


TEXT-FIG. 4. Chart of Table IX showing the effect of heat on the pancreatic perfusates. The upper curve shows the utilization of dextrose in the experiments in which the pancreatic perfusate was heated to boiling and cooled before perfusion through the heart, while the lower curve shows what occurred when the pancreatic perfusate was only heated to 56°C. for 30 minutes.

TABLE X.

Results of Experiments to Determine the Amount of Pancreatic Perfusate Necessary to Enable the Heart to Utilize Dextrose Immediately.

Experiment No.	Pancreas perfused 30 min. Perfusate removed and apparatus sterilized. Heart then perfused with the following mixtures.	Dextrose utilized by pancreas.	Weight of heart.	Dextrose after 10 min. heart perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.
					Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	
			gm.	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*
49	Pancreatic perfusate 10 cc. Locke's solution 240 "	0	70	0.265	0.176	3.07	0	0.173	0.122	0	0.165	0.244	0	0.149	0.49	0	0
50	Pancreatic perfusate 10 " Locke's solution 240 "	0	119	0.284	0.175	1.76	0	0.157	0.27	0	0.158	0	0	0.158	0	0	0.248
57	Pancreatic perfusate 5 " Locke's solution 245 "	0	83	0.200	0.153	1.41	27	0.143	0.316	5	0.142	0.011	25	0.141	0.032	128	0.083
59	Pancreatic perfusate 1 " Locke's solution 249 "	0	75	0.200	0.182	0.564	0	0.183	0	0	0.166	0.50		0.154	0.37	89	0



TEXT-FIG. 5. Chart of Table X showing the result of adding different amounts of the pancreatic perfusate to a perfusion of the heart from the same dog.

Text-fig. 5. The pancreas was first perfused with 250 cc. of Locke's solution containing 0.2 per cent of dextrose. This perfusate was then removed and the apparatus was washed and sterilized for 30 minutes to inactivate any adherent pancreatic substance. The heart of the same animal was then perfused with a fresh solution to which varying amounts of the pancreatic perfusate had been added, the total volume of the perfusate being made up to 250 cc. As seen in the curves of Text-fig. 5, when 10 cc. of the pancreatic perfusate were added, the curve of dextrose utilization by the heart is practically the same as if the entire 250 cc. had been used. Hydrolysis of the final perfusates, however, did not give as definite an increase in reducing substance as in the typical heart-pancreas perfusions. When 5 cc. and 1 cc. were used, though the utilization of dextrose began within the 1st hour, it did not proceed as rapidly as when the larger amounts were used. These experiments indicate that even 1 cc. of a 250 cc. pancreatic perfusate added to 249 cc. of Locke's solution has a distinct effect in enabling the heart to utilize dextrose immediately.

Instability of the Pancreatic Perfusate after Perfusion.

It was hoped to carry out a number of other experiments which would depend on the ability of the pancreatic perfusate to maintain its activity when kept for 24 hours or more without a preservative. To test this, three experiments were carried out. The figures are shown in Table XI. A pancreas was perfused with 250 cc. of Locke's solution for 30 minutes and the perfusate was placed on ice for 48 hours in one experiment and 24 hours in two experiments. At the end of that time the heart of another dog was perfused with the entire pancreatic perfusate, but with the result that only the heart type of dextrose utilization occurred. It hardly seemed probable that this could be due to the fact that different dogs were used, but rather that the enzyme-like substance had lost its activity during these short periods of time. That the perfusates used were originally fully active was demonstrated by the fact that they were the perfusates which had previously been shown to be highly active.

TABLE XI.

Experiments to Determine whether the Pancreatic Perfusate when Allowed to Stand in the Ice Box Loses Its Power to Enable the Heart to Utilize Dextrose Immediately.

Experiment No.	Heart perfused with pancreatic perfusate from experiment No.	Pancreatic perfusate in ice box.	Weight of heart.	Dextrose after 10 min. heart perfusion.	1st hr.			2nd hr.			3rd hr.			4th hr.			Dextrose per gm. of heart recovered by hydrolysis.
					Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	Dextrose.	Dextrose disappeared per gm. of heart.	Bacteria per cc.	
		hrs.	*cm.*	*per cent*	*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*	*mg.*		*mg.*
56	54	48	59	0.200	0.200	0	4	0.184	0.27	28	0.166	0.26	344	0.151	0.18	824	0
58	57	24	56	0.200	0.200	0	980	0.200	0	220	0.167	1.33	312	0.148	0.777	920	0
60	59	24	54	0.195	0.191	0.18	54	0.183	0.32	12	0.154	1.22	9	0.138	0.69	44	0

The Latency of Sugar Utilization.

An interesting finding which should be mentioned is the fact that in the heart-pancreas perfusions no dextrose utilization ever seemed to have occurred in the first 10 minutes of perfusion, the period which was allowed to elapse before filling the control apparatus. If the dextrose had been used by the heart in this time at the same rate that it was used in the following hour, the utilization should at least have been evident. Even in the experiments where the pancreas was first perfused and the heart subsequently perfused with the same fluid, there was no evident utilization of sugar during the first 10 minutes. In such experiments the hearts must have been supplied immediately by an abundance of the pancreatic enzyme. From my experiments it cannot be stated how much more than 10 minutes elapsed before utilization of dextrose began, for an hour was allowed to pass before making another reading. It would seem, therefore, that there is a certain latent period before the heart begins to use dextrose, even though the dextrose is accompanied by an abundance of the pancreatic enzyme.

SUMMARY.

The experiments indicate that the pancreas, when perfused aseptically with Locke's solution containing physiological concentrations of dextrose, does not alter the reducing properties of the perfused solution. The pancreas, however, seems to supply something to the Locke's solution circulating through its arteries which in some way brings about a utilization of sugar by the living heart to an extent that does not occur with the heart alone. This pancreatic substance possesses some of the characteristics of an enzyme. It is inactivated by boiling; it is unstable, rapidly becoming inactive on standing; it acts in small amounts; it causes a great acceleration in the rate of a reaction which otherwise proceeds slowly, and the rate of reaction diminishes as the reaction proceeds. Thus this substance has more of the characteristics of an enzyme than of a stable internal secretion like that of the adrenal glands. The disappearance of sugar was dependent upon the presence of living heart tissue, and it ceased as soon as the perfusate was removed from the heart-pancreas circula-

tion and did not occur at all when a pancreatic perfusate was passed through a non-beating heart. This result indicates that the reaction is not similar to that obtained when muscle and pancreas extracts act on more concentrated solutions of dextrose.

The living heart in the presence of the pancreatic factor and dextrose, is responsible for two effects. First, a condensation of the sugar to a non-reducing form that yields again a simple sugar on hydrolysis or by simply standing, with a preservative, at 37°C. for 24 hours. Second, a disappearance of sugar which is probably due to its destruction by hydrolysis or oxidation. After deducting the reducing sugar in the heart-pancreas perfusions which could be recovered by hydrolysis, the amount of sugar which had actually disappeared exceeded that which was used by the heart when perfused with dextrose alone. As to the fate of this portion of the sugar, no definite evidence was obtained.

The question arises as to whether this substance obtained from the perfused pancreas is identical with the hypothetical internal secretion of the pancreas so essential in sugar metabolism. That there is an internal secretion of the pancreas which can be obtained by this method, and that in some way it accelerates the utilization of sugar by the living heart, seems evident. Though the conclusions are based on the heart and pancreas isolated from the numerous interrelating factors occurring in the body, the evidence suggests, at least, that the substance or substances obtained by perfusing the pancreas may be concerned in the normal activity of the pancreas upon sugar metabolism.

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A CONTRIBUTION TO THE EPIDEMIOLOGY OF LOBAR PNEUMONIA.

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Statistics demonstrate that of all the acute infectious diseases in the United States, lobar pneumonia is responsible for the greatest number of deaths. The incidence of this disease in the larger cities during the winter months is extraordinarily high, and health reports indicate that during the past three decades there has been but little evidence of a tendency to a spontaneous decrease in its morbidity. Scientific investigation of pneumonia has dealt largely with methods of therapy, and but little or no effort has been expended in the elaboration of means of prevention. In recent years the study of the bacteriology of lobar pneumonia has developed new facts, which make it advisable to study the epidemiology of the disease, in the hope that such an investigation may bring to light important factors concerned in its transmission from one individual to another.

Dochez and Avery[1] have reported observations which suggest that in a majority of instances of lobar pneumonia, the infection is acquired by transmission of the infectious agent from a recovered case which still harbors in the mouth secretions the organism responsible for the disease, or from a healthy carrier of pathogenic pneumococci. The validity of this assumption depends upon the following factors. Lobar pneumonia in 65 per cent of cases is due to specific types of pneumococci possessed of high pathogenicity, which do not occur in the buccal secretions of normal individuals, except in instances where there has been intimate association with persons suffering from the disease. Although pneumococci are present in the mouths of 60 per cent of normal individuals, the organisms present are readily

[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1915, xxii, 105.

distinguishable from the highly parasitic types of pneumococcus which cause the severe forms of lobar pneumonia. The organisms found in normal mouths, moreover, occasion but a relatively small percentage of the total cases of this disease.

In the present paper are reported the facts obtained during an investigation, which included, first, a study of the varieties of pneumococci concerned in the production of cases of lobar pneumonia admitted to the Hospital of The Rockefeller Institute during the past 4 years; second, a study of the varieties of pneumococci in the mouths of normal individuals; third, the frequency of occurrence of disease-producing types of pneumococci in the mouths of normal individuals; fourth, the frequency of the occurrence of disease-producing types of pneumococci in the mouth secretions of healthy persons associated with cases of lobar pneumonia; and fifth, a study to determine the period of time during which convalescents harbor in the mouth the types of pneumococci responsible for the disease.

Varieties of Pneumococci Concerned in the Production of Lobar Pneumonia.

During the past 4 years a careful study has been carried on of the types of pneumococci isolated from cases of lobar pneumonia admitted to the wards of the Hospital of The Rockefeller Institute. These organisms have been isolated from the sputum, blood, or lung directly, and in many instances from more than one of these sources, and classified according to the biologic groups previously described.[2] The results of this study are shown in the following table.

TABLE I.

Types of Pneumococci Causing Lobar Pneumonia.

Types.	1912–13	1913–14	1914–15	1915–16	Total.	Per cent.
I	29	22	21	33	105	33.54
II	8	28	35	28	99	31.62
III	7	6	9	13	35	11.18
IV	16	13	17	28	74	23.64

[2] Dochez, A. R., and Gillespie, L. J., *J. Am. Med. Assn.*, 1913, lxi, 727. Dochez and Avery, *J. Exp. Med.*, 1915, xxi, 114.

During the year 1915–16 the incidence of Types II a, II b, II x, subvarieties of Group II described by Avery,[3] has also been studied. In 102 cases of lobar pneumonia Type II a occurred five times, Type II b not at all, and Type II x once.

Examination of Table I reveals the constancy with which the various types of pneumococci occur from year to year. In the years 1912–13 and 1915–16 Type I was the dominant one. In 1913–14 and 1914–15 the percentage incidence of Type II increased, making this group the dominant type during these years. The percentage incidence of cases due to Types III and IV has remained fairly constant from year to year. Inasmuch as it has been previously demonstrated that organisms of the various types differ in their virulence for human beings, changes in mortality from year to year may be dependent upon the dominance of a given type. Of the 313 cases of lobar pneumonia presented in Table I, 204, or 65 per cent, were caused by pneumococci belonging to Types I and II. Since these types are not found in the normal mouth except in the case of carriers, they are regarded as strictly disease-producing organisms, and infection with organisms of either type probably occurs only as a result of direct or indirect contact with a case of pneumonia infected with an organism of one of these types. Recent studies show that pneumococci of Type III are fairly common in the mouth flora of healthy individuals and infections with organisms of this type may be autogenic in nature. The atypical organisms belonging to Type II which induce lobar pneumonia are indistinguishable from the similar organisms which may be the dominant type of pneumococcus found in the normal mouth, and infection with these organisms may also be autogenic.

Varieties of Pneumococcus in the Normal Mouth.

The pneumococcus occurs in the mouth secretions of normal individuals in a large percentage of cases. In some individuals the organism may be present constantly in the mouth secretions, while in others it may be present at one time and absent at another. In

[3] Avery, O. T., *J. Exp. Med.*, 1915, xxii, 804.

Table II is presented a study of types of pneumococcus recovered from the saliva of 398 healthy persons.

TABLE II.

Types of Pneumococcus Isolated from the Sputum of Normal Individuals.

Pneumococcus.	Incidence.	Per cent.
Type I	4	2.16
" II	4	2.16
" II a	0	
" II b	11	5.84
" II x	15	7.79
" III	44	23.40
" IV	110	58.51
Pneumococcus present.	172	
" absent.	226	
	398	

Among 398 normal individuals examined pneumococcus was obtained in 172 instances. In 226 individuals no pneumococcus could be demonstrated in the mouth at the time of examination. In four instances the pneumococcus isolated was Type I. In three of these individuals a history of close association with a case of lobar pneumonia was obtained, whereas in one no such association could be traced. From four individuals, organisms of Type II were obtained. Three of these individuals gave a history of intimate contact with cases of lobar pneumonia of the same type, and in one no such association could be established. Atypical Type II organisms were isolated twenty-six times. Pneumococci of Type II b were found eleven times and of Type II x fifteen times. In view of the relative frequency with which these atypical Type II organisms can be obtained from the sputum of normal persons who give no history of association with individuals suffering from lobar pneumonia due to similar types, these organisms, like those of Group IV, must be considered as readily adapting themselves to existence in the normal mouth secretions.

Of the 398 normal individuals examined, in no instance was a pneumococcus of Subgroup II a isolated, while this organism was found in five cases of pneumonia.

At the time of publication of previous papers dealing with the epidemiology of lobar pneumonia, biologic studies dealing with the organism of Type III had not been carried sufficiently far to determine whether organisms of this type isolated from normal mouths differed in their immunological reactions from those of the same type obtained from cases of lobar pneumonia. In the present study of 398 normal individuals, the pneumococcus of Type III (*Pneumococcus mucosus*) was present in the sputum in 44 instances. Through the courtesy of the New York State Board of Health we have recently obtained an immune serum which both agglutinates and protects animals against *Pneumococcus mucosus*. With this serum a study of the immunological properties of all the cases of this type of organism which have been isolated from normal mouths, as well as of those which have been isolated from cases of pneumonia, has been made and no biologic differences have been detected. A serum prepared by the immunization of a horse to *Pneumococcus mucosus* isolated from a case of lobar pneumonia agglutinates and protects animals against infection from all strains of this type isolated from patients with pneumonia as well as almost all those isolated from normal persons. Curiously enough, therefore, this organism, which is to be considered as one of the fixed biologic types and which possesses for human beings the greatest virulence of all pneumococci, seems to be able to accommodate itself to saprophytic existence in the normal mouth for long periods of time without producing disease. Although virulent organisms of this type occur in normal sputum with considerable frequency, they are responsible for a relatively small number of cases of lobar pneumonia. Table III shows how long certain normal individuals may harbor *Pneumococcus mucosus* in their mouths without suffering from any recognizable infection.

Table III shows that *Pneumococcus mucosus* may persist in the mouth secretions of normal individuals over long periods of time. The longest interval noted in the present study was over 3 months, and evidence indicates that in some instances this organism may be harbored in the sputum for a year or more with impunity.

Previous studies indicate that the commonest type of pneumococcus found in the normal mouth is Type IV, and in this study of 398 healthy persons organisms of this type were found 110 times.

TABLE III.

Persistence of Pneumococcus mucosus (Type III) in Normal Mouths.

Case No.	Interval.	Type of pneumococcus.
1	1st examination.	III
	4 days.	III
	8 "	III
	22 "	III
	28 "	III
	35 "	III
	42 "	III
	49 "	III
	56 "	No pneumococcus.
	66 "	III
	71 "	No pneumococcus.
	78 "	III
	98 "	III
2	1st examination.	III
	7 days.	No pneumococcus.
	14 "	III
	21 "	III
	49 "	III
	56 "	No pneumococcus.
3	1st examination.	III
	7 days.	III
	14 "	III
	21 "	III
	49 "	III
	77 "	III
	91 "	III
4	1st examination.	III
	15 days.	III
	22 "	III
	29 "	No pneumococcus.
	36 "	III

The technique of isolating the pneumococcus from the mouth secretions of healthy individuals has been that commonly employed as a routine procedure in the isolation of pneumococci from the sputum of pneumonia patients.

The saliva of the individuals studied was injected into the peritoneal cavity of white mice, and the pneumococcus was recovered at autopsy from the peritoneal exudate or heart's blood. In order to determine whether the pneumococcus could be cultivated as readily

from the direct sputum culture on blood agar, and whether the pneumococcus thus recovered differed from the organism isolated by animal passage, film preparations of 116 specimens of sputum were made on the surface of blood agar plates, as well as injected intraperitoneally into mice. In 58 specimens pneumococci were neither obtained from the mouse nor from the direct culture. In 47 instances pneumococci were recovered by mouse passage, whereas none was obtained by direct culture. In only 11 instances were pneumococci isolated both by animal inoculation and direct sputum culture, and in each of these cases the type obtained by mouse passage corresponded with that obtained by direct culture.

Incidence of Carrier Condition in Healthy Individuals in Contact with Patients Suffering from Lobar Pneumonia.

Dochez and Avery[4] have shown that a considerable percentage of individuals coming intimately in contact with active cases of lobar pneumonia due to infection with pneumococci of Type I or Type II, carry in their mouth secretions organisms of the same type as that causing the infection. Table IV presents a study of the types of pneumococci found in the mouth secretions of persons in intimate association with certain of the cases of lobar pneumonia admitted to the Hospital of The Rockefeller Institute during the years 1915–16. These include all the cases in which contacts were examined from this point of view.

Table IV shows the incidence and type of pneumococci found among the associates of the cases of lobar pneumonia. In 24 of these cases infection was due to pneumococci of Type I, and in 17 to pneumococci of Type II. In the normal mouth secretions of 44 contact cases infected with Type I pneumococci, pneumococci of a similar type were found in two instances, 8.33 per cent of such cases showing at least one contact among the associates. In all, 44 individuals in contact with pneumonia cases infected with Type I pneumococci were studied, and 4.5 per cent of these persons were found to harbor in their saliva pneumococci of a similar type. Among the associates of the 17 cases infected with Type II pneumococci, three

[4] Dochez and Avery, *J. Exp. Med.*, 1915, xxi, 114.

TABLE IV.

Incidence of Carrier Condition in Healthy Individuals in Contact with Lobar Pneumonia.

Case No.	Type of infecting pneumococcus.	Relationship of associates.	Day of disease.	Type found in associates.
2,443	II	Sister.	2	II and IV
		"	2	II b
2,448	II	Wife.	3	No pneumococcus.
2,456	I	Sister.	29	IV
2,460	I	"	5	III
		"	5	No pneumococcus.
		Brother.	5	II b
2,477	II	"	6	IV
		"	26	IV
		"	22	III
		Sister.	6	III
		"	13	II x and IV
2,493	II	Brother.	4	II
		"	3	IV
		"	3	III
		"	28	No pneumococcus.
		Sister.	4	" "
		"	4	" "
2,497	I	Wife.	2	IV
		Brother.	5	IV
2,519	II	"	8	No pneumococcus.
		"	14	" "
2,521	II	Wife.	7	II
2,524	II	Sister.	9	No pneumococcus.
		"	11	IV
2,527	I	Husband.	8	No pneumococcus.
		"	9	IV
2,537	II	Mother.	5	IV
		"	6	IV
		"	13	No pneumococcus.
		"	18	" "
		Father.	4	" "
		"	18	II x
2,551	I	Wife.	5	No pneumococcus.
		"	10	" "
2,553	I	Mother.	4	" "
		"	9	" "
		"	16	" "
		Sister.	9	II b
		"	16	No pneumococcus.
2,556	I	Wife.	8	" "

TABLE IV—*Continued.*

Case No.	Type of infecting pneumococcus.	Relationship of associates.	Day of disease.	Type found in associates.
2,557	I	Mother.	4	No pneumococcus.
		Sister.	9	" "
2,558	I	Wife.	8	IV
		"	13	IV
		"	21	IV
2,560	I	Mother.	11	No pneumococcus.
2,571	I	Brother.	3	IV
		Sister.	3	IV
		"	16	I
2,572	II	Daughter.	9	IV
		Son.	10	IV
2,573	II	Brother.	2	IV
		Father.	20	No pneumococcus.
		Mother.	21	" "
2,576	I	Wife.	3	IV
		"	17	No pneumococcus.
		Sister.	24	III and IV
		"	24	No pneumococcus.
2,581	II	Wife.	10	IV
		Sister.	16	IV
2,586	II	Mother.	3	IV
		Father.	3	No pneumococcus.
		"	15	IV
		Brother.	8	III
		Sister.	8	IV
		"	15	IV
		"	22	No pneumococcus.
2,587	I	Husband.	6	II x
2,589	I	Wife.	12	IV
		"	19	IV
2,595	I	Sister.	6	No pneumococcus.
		Wife.	6	II
		"	9	II
		"	17	II
		"	23	II
		"	25	II
		"	31	II
		"	33	II
		"	34	II and III
		"	38	II
		"	39	II
2,597	I	Sister.	11	No pneumococcus.
		Brother.	18	" "

TABLE IV—*Continued.*

Case No.	Type of infecting pneumococcus.	Relationship of associates.	Day of disease.	Type found in associates.
2,602	I	Cousin.	6	No pneumococcus.
		Mother.	5	IV
		"	11	IV
2,604	II a	Sister.	18	III
		Brother.	11	II x
2,608	I	Wife.	5	III
		"	16	III
		"	24	III
		"	52	No pneumococcus.
		"	65	IV
		Brother-in-law.	52	No pneumococcus.
		Sister.	59	III and IV
2,611	II	"	1	II x
		"	22	II x
		Wife.	2	II b and III
		"	16	III
		"	36	III and IV
		Daughter.	1	IV
		Niece.	22	No pneumococcus.
2,615	II	Sister.	2	IV
		"	13	No pneumococcus.
		Brother.	7	" "
		"	13	IV
		Wife.	2	No pneumococcus.
		"	21	III
		"	27	No pneumococcus.
2,622	II	Daughter.	12	" "
		Husband.	14	" "
		Daughter.	85	" "
2,623	I	Wife.	5	" "
		"	9	II b
		"	17	II b
		"	23	II b
		"	30	IV
		"	38	II b
		Nephew.	23	III
		Niece.	23	No pneumococcus.
		Friend.	23	" "
2,625	I	Sister.	15	IV
2,626	II	"	10	No pneumococcus.
		Father.	26	II b

TABLE IV—*Concluded.*

Case No.	Type of infecting pneumococcus.	Relationship of associates.	Day of disease.	Type found in associates.
2,630	I	Father.	2	No pneumococcus.
		Mother.	9	" "
		"	16	" "
		"	24	" "
		"	32	" "
2,632	I	"	25	" "
		Father.	25	IV
		Sister.	37	IV
2,647	I	Wife.	2	No pneumococcus.
		Sister.	6	IV
		"	20	IV
2,652	I	Mother.	10	I

Summary.

Type.	No. of pneumonia cases studied.	No. of cases giving rise to carriers.	Per cent.	Total contacts examined.	No. of positive contacts.	Per cent.
I.....	24	2	8.33	44	2	4.5
II.....	17	3	17.64	40	3	7.5
Total.....	41	5	12.19	84	5	5.95

(17.64 per cent) were found to harbor in their mouths pneumococci of Type II. Altogether 40 contacts of cases due to Type II pneumococci were examined and from the sputum of 7.5 per cent of these, pneumococci of Type II were isolated. From a total of 41 cases of lobar pneumonia due to Type I and Type II, 5 gave rise to the carrier condition in at least one of their immediate associates, 12.19 per cent. The total number of contacts examined was 84, and of these 5, or 5.95 per cent, showed either pneumococci of Type I or Type II in their sputum. In one instance pneumococci of Type II were isolated from the sputum of the wife of a patient who suffered from pneumonia due to pneumococci of Type I. This is the only instance in our experience in which a pneumococcus of Type I or Type II has been isolated from the mouth of a contact where the type of organism found in the contact has differed from that isolated

from the patient. It is of interest in connection with this example that during convalescence the Type I pneumococcus disappeared from the sputum of the patient, and a pneumococcus of Type II then appeared in his mouth flora. A possible explanation is that the wife was a chronic carrier of Type II pneumococcus and that during convalescence, during which period the patient was in close contact with his wife, he secondarily acquired this type from her.

In this table no study of the carrier condition in associates of patients of Type III is recorded. Dochez and Avery in their report assumed the Type III pneumococcus to be a strictly disease-producing organism and not an inhabitant of the normal mouth secretions. Subsequent studies have shown that pneumococci of Type III are found so frequently in the mouths of normal individuals who have not associated with cases of lobar pneumonia that pneumococci of this type must be regarded as normal inhabitants of the healthy mouth. In view of these findings it would seem that the occurrence of this type of pneumococci in the mouths of individuals associated with cases of lobar pneumonia due to other types is to be expected and cannot be regarded as evidence against the epidemiological significance of the occurrence of carriers of pneumococci of Types I and II.

In this table are shown the results of repeated observations of the types of pneumococci in the mouths of a number of normal individuals. These observations bring to light the facts that the sputum type of pneumococcus may be present at one time and absent at another, that the types of pneumococci present may change from time to time, and also that more than one variety of sputum pneumococcus may be present at the same time.

Persistence during Convalescence of the Type of Pneumococcus Causing the Disease.

Previous study has demonstrated the fact that during the period of recovery from lobar pneumonia the type of pneumococcus causing the disease disappears from the mouth flora and in many instances is subsequently replaced by a pneumococcus of the variety commonly found in normal sputum. These facts are further corroborated by the evidence presented in Table V.

TABLE V.

Persistence during Convalescence of the Type of Pneumococcus Causing the Disease.

Case No.	Type of pneumococcus during height of disease.	Day of disease.	Type of pneumococcus recovered.
2,456	I	14	No pneumococcus.
		27	" "
2,464	I	31	" "
2,465	III	11	III
		13	III
		20	II x
		27	IV
		34	II x and IV
		40	II x and IV
2,477	II	6	II
		13	II b
		21	II b
		29	II b and IV
2,488	III	4	III
		14	IV
		21	IV
		28	IV
2,493	II	5	II
		15	III
		26	IV
2,497	I	2	I
2,519	II	9	II
		16	No pneumococcus.
2,521	II	20	" "
		27	" "
2,524	II	8	II
		15	II x
		22	No pneumococcus.
2,527	I	10	I
		24	No pneumococcus.
2,536	II	10	II
		17	No pneumococcus.
		25	" "
2,543	II	11	" "
		18	" "
		31	IV
2,547	II	20	No pneumococcus.
2,549	II a	19	" "
		26	" "
2,550	III	14	III
		21	IV

TABLE V—*Continued.*

Case No.	Type of pneumococcus during height of disease.	Day of disease.	Type of pneumococcus recovered.
2,551	I	6	I
		25	II a
		32	II a
2,553	I	17	No pneumococcus.
		24	" "
		31	" "
2,556	I	9	IV
		16	No pneumococcus.
2,557	I	10	IV
		17	No pneumococcus.
		24	II a
2,558	I	21	No pneumococcus.
		28	" "
2,560	I	11	IV
		18	II x
		25	No pneumococcus.
2,563	II a	12	IV
		26	No pneumococcus.
2,566	III	8	III
		15	III
2,571	I	9	No pneumococcus.
		16	IV
		23	IV
2,572	II	10	IV
		17	IV
		24	No pneumococcus.
		31	" "
		45	" "
2,573	II	19	" "
		26	" "
		40	II and IV
		47	IV
		57	IV
		64	No pneumococcus.
		71	II
		78	No pneumococcus.
		85	II
2,576	I	4	No pneumococcus.
		11	I
		18	No pneumococcus.

TABLE V—*Continued.*

Case No.	Type of pneumococcus during height of disease.	Day of disease.	Type of pneumococcus recovered.
2,615	II	7	II
		14	No pneumococcus.
		21	II
		28	II
2,622	II	16	II x
		23	IV
		32	II x
		39	II x
		53	No pneumococcus.
		60	II x
		72	II x
		79	II x
		86	II x
		100	No pneumococcus.
2,623	I	17	I
		24	No pneumococcus.
		31	I
		38	I
		45	I
2,625	I	8	I
		15	No pneumococcus.
2,626	II	11	II
		18	II
		25	II
2,630	I	3	I
		17	II x and IV
		24	II x
		38	II x
2,632	I	23	I and IV
		37	II x
2,633	I	4	I
		11	I
		19	I
2,689	III	7	III
		14	III
		21	III
		28	III
		35	III
2,647	I	6	No pneumococcus.
		13	" "
		20	I

TABLE V—*Continued.*

Case No.	Type of pneumococcus during height of disease.	Day of disease.	Type of pneumococcus recovered.
2,640	III	7	No pneumococcus.
		14	" "
		21	" "
		24	" "
		28	" "
2,581	II	4	II
		11	No pneumococcus.
		18	" "
		25	II
		32	No pneumococcus.
		47	" "
2,582	III	5	III
		12	III
		26	No pneumococcus.
2,586	II	8	" "
		22	" "
2,587	I	7	" "
		14	IV
		21	No pneumococcus.
		28	I
		35	No pneumococcus.
		42	" "
2,589	I	6	I
		12	I
		19	No pneumococcus.
		26	IV
2,593	III	3	III
2,595	I	16	No pneumococcus.
		23	II
		30	II
2,597	I	11	III and IV
		18	II x, III, and IV
		25	IV
2,602	I	12	IV
		18	IV
		25	IV
2,604	II a	12	III
		19	III
2,608	I	14	IV
		21	IV
		28	No pneumococcus.
		35	I

TABLE V—*Concluded.*

Case No.	Type of pneumococcus during height of disease.	Day of disease.	Type of pneumococcus recovered.
2,608	I	42	No pneumococcus.
		49	" "
		56	" "
		63	II x
		77	No pneumococcus.
		84	" "
2,611	II	2	II
		9	IV
		16	IV
		23	IV
		30	No pneumococcus.
		37	IV
2,652	I	13	I
		20	I
		27	I
		53	No pneumococcus.
		60	" "
		67	" "
		74	III
		81	No pneumococcus.
		88	" "
		95	" "

Table V records the results of a study of the length of time during which the type of pneumococcus responsible for the disease could be isolated from the sputum of 53 cases of lobar pneumonia due to Types I, II, and III. The carrier condition in the convalescent as measured from the onset of the disease is, on the whole, relatively brief.

The shortest period of carrying was 7 days, the longest 85 days. The long persistence of the disease strain may be associated with delayed resolution of the lung lesion or the occurrence of some complication. It is further evident from this study that the type organism may disappear or fail to be isolated from the mouth secretions of the recovered case at a given examination, and then be found again at a subsequent period. In only one instance during convalescence has a Type II pneumococcus been recovered from a patient convalescent from a Type I pneumonia. In this case the patient's wife

was a chronic carrier of the pneumococcus of Type II. A Type I pneumococcus has never been recovered from a patient convalescent from a Type II pneumonia.

DISCUSSION.

The results of the work detailed in the present report in general confirm the previous observations of Dochez and Avery on the relationship of the varieties of pneumococcus to lobar pneumonia and the occurrence of carriers of disease-producing types of pneumococci. Combination of the results of study over a period of years of the types of pneumococci inducing lobar pneumonia shows that the majority of instances of infection are due to organisms belonging to Types I and II. A small number of cases are due to infection with atypical pneumococci of Type II, as described by Avery, and of these organisms those belonging in the so called Subgroup II a are responsible for the greatest number of cases. Pneumococci of Type III in spite of their high virulence for human beings are responsible for the smallest percentage of cases of lobar pneumonia. The incidence of infection with pneumococci of Type IV seems to remain fairly constant from year to year. As the different types of pneumococci show differences in virulence for human beings, it is possible that variations in the mortality rate from year to year may be directly related to the seasonal dominance of different types.

Comparison of the pneumococci obtained from the mouth secretions of normal individuals with those isolated from diseased persons shows the existence of two general classes of organisms. One of these, consisting of pneumococci of Types I and II, occurs only in association with disease; the other, including Types III and IV, together with the atypical Type II organisms, causes a minority of the instances of disease, but includes the organisms which are commonly found in normal healthy mouths. In a small number of instances organisms of Types I and II have been found in mouth secretions of normal individuals. In all cases except two, however, in which this has occurred, it has been possible to trace direct contact of the individual with a case of lobar pneumonia caused by the same type of pneumococci. As a large number of normal persons have been examined, it is not surprising that in a few individuals in whom no

association with cases of lobar pneumonia could be traced organisms of the fixed Types I and II should have been discovered.

Among the types of pneumococcus which seem to adapt themselves readily to the normal mouth, Type III presents a paradox. This organism has the biologic characteristics that distinguish Type I and Type II from the other varieties of pneumococcus. All strains of these organisms with but few exceptions have common immunological characteristics. Although this organism is responsible for but a small percentage of the cases of lobar pneumonia, it produces an unusually severe type of the disease. Notwithstanding these facts, this organism is commonly met with in the mouth secretions of normal individuals where it may persist for long periods of time without producing pathological lesions. The strains of this group isolated from normal sputum possess immunological reactions identical with those of the strain isolated from cases of lobar pneumonia. It is of interest, however, that in a study of the mouth flora of cases of pneumonia due to *Pneumococcus mucosus* it has been found that this organism tends to disappear as rapidly from the mouths of patients as do the disease-producing Types I and II. The study of the varieties of pneumococci in the mouths of normal individuals and convalescents from lobar pneumonia shows that in such individuals the type of pneumococcus present may vary from time to time, and also that more than one type may be present at any given time. This suggests the possibility that the sputum types of pneumococci may be passed from one individual to another, and that occasionally an individual may acquire pneumococci of this class which possess sufficient pathogenicity to initiate disease.

These facts suggest the following conclusion concerning the epidemiology of lobar pneumonia. Infection with pneumococci of Type I and Type II must be regarded as dependent upon contact with a previous case of lobar pneumonia due to the same type of organism. These types of infection are either acquired by direct contact with an active case of pneumonia or by association with a healthy carrier of one of these types of pneumococcus, or with a convalescent from lobar pneumonia who still harbors in his mouth secretions the organism responsible for the disease. Infection with the sputum types of pneumococcus, namely, Types III and IV and the atypical strains

of Type II, may be autogenic in character, or due to the acquisition by the individual of a given race of one of these types to which he is especially susceptible.

SUMMARY.

1. Pneumococci of Type I and Type II are responsible for the majority of the cases of lobar pneumonia.

2. Among the pneumococci found in the mouths of healthy individuals, Type IV predominates, Type III is fairly frequent, and atypical organisms of Type II are occasionally encountered. Organisms of these types give rise to a minority of cases of lobar pneumonia.

3. Healthy persons intimately associated with cases of lobar pneumonia may harbor in their mouth secretions the highly parasitic pneumococci of Types I and II.

4. Occasionally a carrier of Type I or Type II pneumococcus is encountered in whom it is impossible to trace any contact with an infected patient.

5. Convalescents from lobar pneumonia may carry for a considerable period of time the type of pneumococcus with which they were infected.

THE VARIOUS FACTORS OF RESPIRATION IN PERSONS WITH PNEUMOTHORAX.

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Plates 44 and 45.

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In connection with the study of the respiration in experimental pneumonia, recently reported by Newburgh, Means, and Porter,[1] it became desirable to find out what effect a great reduction in the functioning lung surface would have on the various factors of respiration. Since pneumothorax presents this condition, the present research was undertaken. Our observations were made upon four cases in which pneumothorax was being produced for the treatment of pulmonary disease, and upon one case of spontaneous pneumothorax.[2]

The clinical data on the five cases are given below.

Case 1.—B. D., female, age 27 years. Artificial pneumothorax.

The first symptoms of pulmonary tuberculosis began 4 years ago. She lost ground rapidly for a year, in spite of rigid sanatarium treatment, and then injections of nitrogen were begun. She soon began to improve and the treatment has been continued ever since. There have been thirty-four injections in all. The dosage was about 1,000 cc. at first; now the average is 500 cc. In March, 1916, she had no symptoms of phthisis and was leading a normal life. Immediately after an injection she was somewhat short of breath, on exertion only.

Physical examination showed the usual signs of pneumothorax in the left chest. X-ray showed a nearly complete collapse of the left lung (Fig. 1).

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[1] Newburgh, L. H., Means, J. H., and Porter, W. T., *J. Exp. Med.*, 1916, xxiv, 583.

[2] Case 2 was a patient at the House of the Good Samaritan, Boston. We are indebted to the superintendent of that institution for making it possible for us to study the case and for furnishing much valuable information.

Case 2.—M. G., female, age 20 years. Artificial pneumothorax.

The first symptoms of pulmonary tuberculosis began 2½ years ago. She has had sanatarium treatment most of the time since. 3 months ago she had much cough and sputum and was running a high evening temperature. Physical examination at that time showed extensive involvement of the left lung with cavity formation, and very slight signs at the right apex. Collapse of the left lung was then begun. Twelve injections of nitrogen have been made in all. Marked improvement was at once apparent. In November, 1915, physical examination showed signs of pneumothorax over the left chest, and x-ray showed an extensive collapse of the left lung.

Case 3.—S. M. H., female, age 32 years. Spontaneous pneumothorax.

The past history is negative.

10 months ago while dancing she suddenly became short of breath. 2 weeks later a diagnosis of pneumothorax was made. The dyspnea gradually decreased, and in November, 1915, she had slight dyspnea on exertion only. She entered the hospital on November 4, 1915, at which time physical examination showed the signs of pneumothorax over the whole left chest, and x-ray showed an extensive left-sided pneumothorax with slight displacement of the heart toward the right. The right lung showed evidence of tuberculosis.

The Wassermann test was negative. The blood showed slight secondary anemia. The pulse, temperature, and respiration rate were all normal while she was in the hospital.

Case 4.—M. P., female, age 22 years. Artificial pneumothorax.

The patient was believed to have a lung abscess.

The family and past history are unimportant. She entered the Massachusetts General Hospital on June 5, 1916, having previously been a private patient of one of us. The history was that of a productive cough of 7 months' duration with considerable loss of strength, and occasional night sweats. The sputum was purulent and copious and was raised most abundantly in the morning. There had been hemoptysis on several occasions. Physical examination at the time of entry showed an area of dullness in the middle of the left back with crackling râles and dullness, with increased breath sounds at the left top posteriorly.

The Wassermann test on the blood was negative. She occasionally ran a slight fever while in the ward.

An x-ray plate taken April 30, 1916, showed an area of dullness between the second and fourth ribs on the right, which extended across the chest from the lung root to the axillary border. This dullness was of an even consistency except at the borders where there was some mottling. The apices were clear, but there was some thickening about the hilus of each lung.

It was thought that she probably had a lung abscess in the lower part of the right upper lobe, and treatment by artificial pneumothorax was undertaken. The following injections of nitrogen were made while she was in the ward.

	cc.
June 8	600
" 9	500
" 12	650
" 18	900
" 24	300
July 3	700
" 6	800

A partial pneumothorax only was secured, as was shown by a series of x-ray plates (Figs. 2 and 3).

June 11. X-ray showed a small area of pneumothorax extending as high as the fifth rib in the axillary border on the right. Otherwise as previously described.

June 13. X-ray showed the lower lobe fairly well collapsed.

June 19. X-ray showed slightly more pneumothorax than at the last examination.

Case 5.—L. R., male, age 22 years. Artificial pneumothorax.

A case of bilateral pulmonary tuberculosis of 4 months' duration with active signs in the left upper lobe. Gradual loss of weight, fever, hemoptysis, and many tubercle bacilli in the sputum. As the disease was rapidly progressing and a brisk hemoptysis had set in, and since the patient was beginning to have an elevated temperature, it was decided to induce artificial pneumothorax and collapse the left lung (Fig. 4).

After the first two injections the patient raised about 8 ounces of blood during the succeeding 3 days, and it was feared the blood might come from the non-collapsed lung. As no signs of activity were noticeable in the non-collapsed lung, more nitrogen was introduced into the left pleural cavity, and soon after that the blood and sputum ceased.

At the present time the patient has a well collapsed left lung, is up and about every day, with no respiratory embarrassment, no cough or sputum, and has gained in weight.

The injections of nitrogen in the case were as follows:

	cc.
April 8	750
" 12	750
" 20	700
May 5	600
June 2	500
" 12	600

Observations with the Unit Apparatus.

Experiments with Benedict's Unit Apparatus (spirometer type, subjects breathing through a mouthpiece) were done with Cases 1, 2, and 3, in the fasting condition, lying prone, at complete rest. The data are given in Table I.

TABLE I.

Experiments with the Unit Apparatus.

Period.	Carbon dioxide per min.	Oxygen per min.	Respiratory quotient.	Ventilation per min. (reduced).	Respiration rate.	Volume per respiration (reduced).	Pulse.	Carbon dioxide in expired air (calculated).
	cc.	*cc.*		*liters*		*cc.*		*per cent*
Case 1. Weight 53.3 kg. Height 174.2 cm. Nov. 8, 1915.								
1	161	210	0.77	5.86	14.8	396	79	2.75
2	160	216	0.74	5.37	12.4	433	76	2.98
3	165	214	0.77	5.25	12.1	433	74	3.14
Average..	162	213	0.76					
Case 2. Weight 57.2 kg. Height 167.5 cm. Nov. 12, 1915.								
1	179	259	0.69	7.06	19.7	358	109	2.54
2	169	236	0.72	6.73	19.4	347	105	2.51
3	171	236	0.72	6.68	19.1	349	102	2.56
Average..	173	244	0.71					
Case 3. Weight 64.5 kg. Height 163.8 cm. Nov. 16, 1915.								
1	184	224	0.82	6.12	14.3	428	87	3.00
2	164	215	0.76	5.77	14.4	400	84	2.84
3	164	217	0.76	5.58	14.2	393	81	2.94
Average..	171	219	0.78					

Basal Metabolism.—This was calculated in each case from the average oxygen absorption and the calorific value of oxygen for the respiratory quotient obtained.[3]

The body surface of each subject was determined by the Du Bois height-weight chart,[4] and the metabolism expressed in terms of the area thus found, was as follows:

Case 1. 37.3 calories per sq. m. per hr.
" 2. 42.0 " " " " " "
" 3. 36.9 " " " " " "

[3] Williams, H. B., Riche, J. A., and Lusk, G., *J. Biol. Chem.*, 1912, xii, 357.
[4] Du Bois, D., and Du Bois, E. F., *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 77.

Alveolar Carbon Dioxide Tension.—In three of the cases of artificial pneumothorax the alveolar carbon dioxide tension was determined by the Plesch-Higgins method. The results were:

	mm.
Case 1	44.3
" 2	39.0
" 5	41.5

Sensitivity of the Respiratory Center.—In three of the cases the sensitivity of the respiratory center was determined by obtaining the reaction to carbon dioxide. The experiment was carried out with the unit apparatus by replacing the absorbers with a large dead space, so that the carbon dioxide gradually accumulated. The curves shown in Tables II, III, and IV were obtained. In Case 4 one curve was secured before and two after the production of pneumothorax.

TABLE II.

Case 1. Reaction to Carbon Dioxide. Subject Lying on Back.

Carbon dioxide in inspired air.	Respiration rate.	Volume per respiration.	Total ventilation per min. (unreduced).	Ventilation coefficient. Increase in total ventilation. Start=100 per cent.
per cent		*cc.*	*liters*	*per cent*
		Feb. 5, 1916.		
0.00	12.0	460	5.51	100
1.42	14.3	481	6.90	125
3.06	13.0	605	7.88	143
3.63	15.3	611	9.36	170
4.66	15.7	765	12.00	217
5.30	15.8	870	13.75	250
5.81	17.0	876	14.90	270
6.50	18.2	975	17.75	322
		Mar. 27.		
0.00	15.5	403	6.25	100
2.58	15.8	536	8.49	136
3.71	16.0	596	9.55	153
4.66	17.5	667	11.69	187
5.70	19.0	741	14.10	226
6.41	19.3	920	17.75	284
7.14	20.0	910	18.20	292
7.60	21.0	1,017	21.35	341

TABLE III.

Case 4. Reaction to Carbon Dioxide. Subject Lying on Back.

Carbon dioxide in inspired air.	Respiration rate.	Volume per respiration.	Total ventilation per min. (unreduced).	Ventilation coefficient. Increase in total ventilation. Start = 100 per cent.
per cent		*cc.*	*liters*	*per cent*
June 6, 1916. Before nitrogen injections had been begun.				
0.00	25.0	336	8.40	100
1.43	25.8	389	10.03	119
3.00	28.3	410	11.60	138
4.40	24.8	556	13.80	164
5.15	24.5	641	15.70	187
5.80	30.5	778	23.73	283
6.60	30.8	1,050	32.35	385
June 14. Has had three injections of nitrogen since last experiment.				
0.00	34.0	284	9.65	100
1.92	35.5	336	11.90	123
3.53	37.0	420	15.2	161
4.52	39.0	525	20.55	212
5.43	39.0	630	24.40	254
6.20	41.0	735	30.615	312
June 30. Two more injections of nitrogen.				
0.00	20.2	420	8.49	100
2.21	25.0	441	11.10	132
4.56	32.5	630	20.50	242
5.21	41.0	630	25.80	304
5.95	44.0	735	32.40	382

TABLE IV.

Case 5. Reaction to Carbon Dioxide. Subject Lying on Back.

Carbon dioxide in inspired air.	Respiration rate.	Volume per respiration.	Total ventilation per min. (unreduced).	Ventilation coefficient. Increase in total ventilation. Start = 100 per cent.
per cent		*cc.*	*liters*	*per cent*
June 12, 1916.				
0.00	23.2	462	10.70	100
2.81	24.5	567	13.90	130
3.96	25.5	777	19.78	185
6.23	32.0	1,177	37.70	352
6.71	36.0	1,240	44.60	417

Vital Capacity.—The vital capacity was secured in Cases 1, 4, and 5.

Case 1 fortunately had had some determinations made of her vital capacity while she was in college, which she was able to obtain for us. These determinations were as follows:

		liters
Oct. 1906	Vital capacity	2.6
" 1907	" "	2.5
	Weight 61 kg.	
" 1910	Vital capacity	2.1
	Weight 63 kg.	

The last was 2 years before the diagnosis of phthisis was made, and she says that she is sure she had tuberculosis at that time, as she distinctly remembers having a slight cough and tiring easily. This fact would explain the decrease in vital capacity.

On February 5, 1916, three determinations of her vital capacity, taken about 5 minutes apart, gave the following results:

	liters
Vital capacity	1.11
" "	1.11
" "	1.26

On March 27, 1916, the vital capacity was 1.5 liters.

The greater value obtained on March 27 is probably due to the fact that the last injection of nitrogen had been 4 weeks before, whereas on February 5, she had had one only 3 weeks before.

In Case 4 one determination was secured before any injections of nitrogen had been made, and two afterward.

On June 6 her vital capacity was 2.74 liters.

On June 14, after she had had three injections of nitrogen, it was 1.36, 1.40, average 1.38 liters.

On June 30, after five injections, it was 1.63 liters.

In other words, although by x-ray only a partial collapse of the right lung was present, yet functionally there was a reduction to one-half her original available ventilating space.

The vital capacity of Case 5 was observed but once. On June 12, it was 1.57 liters, which is less than half what we should expect in a man of medium weight and height.

DISCUSSION.

The observations on these five subjects show that all the factors of respiration are essentially normal in persons with one lung collapsed. The gaseous exchange is normal, for if we calculate the basal metabolism, we get normal values: 37.3 calories per sq. m. per hour for Case 1, 42.0 for Case 2, and 36.9 for Case 3 are all within 10 per cent of the normal average for women of their age. The respiratory quotients are perfectly reasonable except that that for Case 2, of 0.71, is somewhat low. She was, however, the subject with the most active tuberculosis and might easily have an increased catabolism and so reach a fasting respiratory quotient sooner than a normal subject.

The respiration rate, the volume per respiration, and the total ventilation of the lungs, in the three cases in which they were determined, are probably all within normal limits, though possibly the total ventilation in Case 2 is a little higher than is usually found.

The percentage of carbon dioxide in the expired air is within normal limits in Cases 1 and 3, but is somewhat low in Case 2.

The alveolar carbon dioxide by the Plesch method was normal in Case 1 (44.3 mm.) and Case 5 (41.5 mm.), but was somewhat low (39 mm.) in Case 2. This probably explains the low percentage of carbon dioxide in the expired air, and the higher ventilation in the case of the last subject. It may have been due to a very slight acidosis.

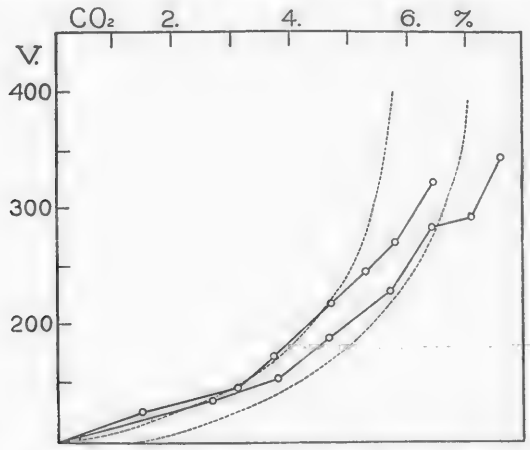
The reactions to increasing amounts of carbon dioxide in all the cases studied in this manner show that up to the point at which the respiration is trebled (at from 5.5 to 6.5 per cent of carbon dioxide in the inspired air) the increase in ventilation falls within the normal zone determined by Peabody,[5] as is shown in Table V. In this table the curves have been simplified by reducing the figures for ventilation increase to correspond to even percentages of carbon dioxide. This was done by plotting the actual observations and drawing the most probable curve through these points, then reading the values at the even percentages. The curves obtained with Case 1 are also shown graphically in Text-fig. 1.

[5] Peabody, F. W., *Arch. Int. Med.*, 1915, xvi, 851.

TABLE V.

Summary of Carbon Dioxide Reactions.

Subject.	Date.	Increase in ventilation to increasing amounts of carbon dioxide in the inspired air. Carbon dioxide. 1 per cent.	2 per cent.	3 per cent.	4 per cent.	5 per cent.	6 per cent.	7 per cent.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Case 1.	Feb. 5	116	131	143	190	235	285	
	Mar. 27	112	126	140	162	200	243	305
Case 4.	June 6	110	125	138	157	190	305	
	" 14	110	125	145	177	225	300	
	" 30	115	130	170	215	285	390	
Case 5.	June 12	112	122	140	190	263	336	455
Reactions of normal persons, taken from Peabody's diagram.		0 to 112	105 to 130	122 to 150	148 to 185	180 to 250	237 to 450	350 and up.



TEXT-FIG. 1. Case 1. Reaction to carbon dioxide. The ordinates show the percentage of carbon dioxide in the inspired air, the abscissæ the ventilation coefficient. The subject's normal ventilation, breathing atmospheric air, is called 100 per cent. At 200 per cent the ventilation is doubled, etc. The dotted lines represent Peabody's group of reactions in normal subjects.

The reaction obtained on February 5 with Case 1 was discontinued at 6.5 per cent of carbon dioxide; that of March 27, however, was continued up to 7.6 per cent carbon dioxide. It is interesting to note that in the latter curve the last two points show a distinct decrease in reaction, a flattening of the curve, while in Peabody's normal cases this was the point at which the curve became steeper. We interpret this finding as meaning that the subject reaches her limit at this point. The normal respiratory apparatus can increase the normal resting ventilation from four- to eightfold without great discomfort. This patient, with one lung, has a lower limit, but up to her limit reacts normally.

In some experiments upon one of us, Newburgh and Means found that the ventilation was trebled when the subject was doing about 500 kg. m. of work per minute.[6] This would be the equivalent in Case 1 of going up about three flights of stairs in 1 minute. As a matter of fact, she can do more than that without urgent dyspnea. At the end of each reaction to carbon dioxide, her volume per respiration was from 65 to 80 per cent of her vital capacity, but the rate had only increased in one experiment 50 per cent, and in the other 35 per cent. It would seem as though a further increase in the ventilation might have been met by an increase in rate, but such was, actually, not the case.

With Cases 4 and 5 much the same thing was found. The curves are both normal as far as they go. Case 4, with only a partial pneumothorax but with a vital capacity reduced to half of what it had been before the injection of nitrogen, was almost able to quadruple her ventilation. Case 5, with a more complete collapse and a vital capacity less than one-half what it should be, was more than able to quadruple his breathing. These functional capacities compare favorably with those of some normal persons.

The vital capacity was definitely reduced in the three cases of artificial pneumothorax, when it was determined.

[6] Means, J. H., and Newburgh, L. H., *J. Pharm. and Exp. Therap.*, 1915, vii, 454.

CONCLUSION.

It may be said that at rest all the factors of respiration, gaseous exchange, carbon dioxide tension, and the mechanical factors, are normal in persons with a collapsed lung, that the reaction to carbon dioxide is normal up to the point at which the respiration is trebled, or sometimes quadrupled, but that beyond that point a limit may be reached.

The ventilation of the lungs can be accomplished in an entirely normal manner in spite of a greatly reduced vital capacity. The only difference between normal persons and persons with a collapsed lung is that the latter when called upon to increase their ventilation reach their limit a little sooner than the former.

From these findings we might deduce that there will be no dyspnea except after moderate exertion. And this deduction is borne out by the histories of the patients.

In other words, in the lungs, as in other organs, there is a large factor of safety, one lung being as efficient as two, except when the work done calls for more than a threefold increase in the normal ventilation.

EXPLANATION OF PLATES.

PLATE 44.

FIG. 1. Case 1. Artificial pneumothorax.
FIG. 2. Case 4. Before nitrogen injections were begun.

PLATE 45.

FIG. 3. Case 4. Artificial pneumothorax.
FIG. 4. Case 5. Artificial pneumothorax.

FIG. 1.

FIG. 2.

(Means and Balboni: Factors of Respiration in Pneumothorax.)

FIG. 3.

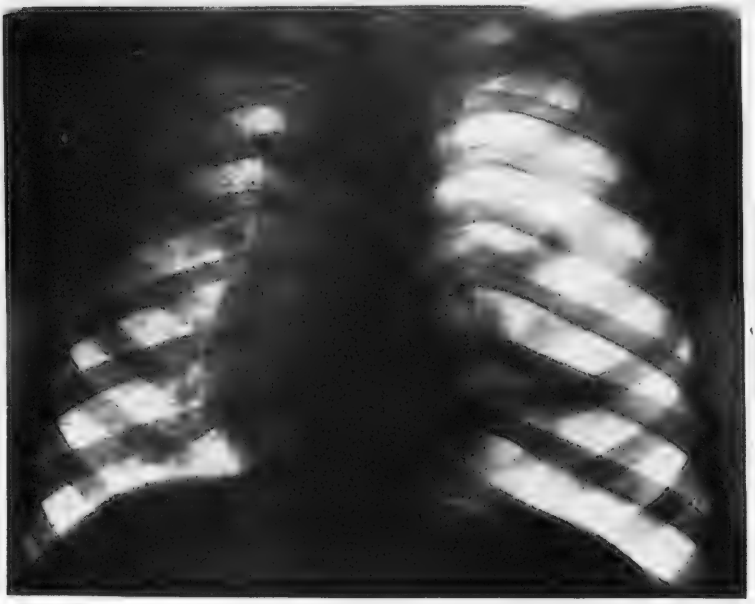


FIG. 4.

(Means and Balboni: Factors of Respiration in Pneumothorax.)

THE COMPARATIVE RESISTANCE OF BACTERIA AND HUMAN TISSUE CELLS TO CERTAIN COMMON ANTISEPTICS.

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(Received for publication, August 19, 1916.)

A number of chemicals are strongly bactericidal even in weak dilution when tested on bacteria suspended in broth cultures or in salt solution. In the presence of serum stronger solutions are usually necessary, while in order to kill pathogenic microorganisms growing in the tissues, as, for example, in infected wounds, the antiseptic must often be applied in such strength that body cells, as well as bacteria, are injured or destroyed.

An ideal antiseptic is obviously one that will kill the infecting agent without at the same time injuring body cells.

It is not practicable to carry out on infected wounds,—in man, at least,—experiments directed toward the discovery of such a substance. It occurred to the author, however, that in tissue cultures conditions might be made to approximate those in the living organism; for bacteria and tissue cells growing together *in vitro* may be easily subjected to the same chemical agents and the effect on each be directly observed.

Experiments were therefore undertaken to investigate the comparative resistance of body tissues (wandering cells and connective tissue cells) to various chemicals, including especially a number of those in common use as antiseptics.

Technique.

Human tissues were used throughout the experiments since it was thought that the results would be of more value if clearly applicable

to human beings. We have shown in a former paper[1] that connective tissue and wandering cells can be cultivated *in vitro* in a modified plasma medium almost as easily as similar tissues of lower animals. Tuberculous and Hodgkin's lymph glands removed at operation[2] and spleens taken out at autopsy a few hours after death were the tissues used. With each of these a migration of large mononuclear cells and connective tissue cells was obtained. The organism used was *Staphylococcus aureus*, chosen first because of the frequent infections caused by it, and, secondly, because it has been shown[3] to occupy a median position among the pathogenic bacteria in its resistance to disinfectants. It is more resistant, for example, than the streptococcus, but less resistant than *Bacillus pyocyaneus*. Staphylococci from two different sources were used; one strain from the throat of an apparently healthy individual, another from a case of furunculosis. The two strains exhibited little difference in their resistance to the chemicals tested.

In a few preliminary experiments the disinfectant or chemical to be tested was added in the desired strength to the plasma medium of the tissue culture. It was found, however, that certain of the substances used, especially iodine and hypochlorites, caused a liquefaction of the fibrin of the clotted plasma so that the cells found no framework upon which to grow and thus the experiment proved nothing as regards the effect of the chemical on cells. Furthermore, the results as regards the destruction of bacteria were also not uniform, probably owing to the imperfect diffusion of the disinfectant through the clotted medium. The limited diffusibility of chemicals through the plasma clot of tissue cultures has been well demonstrated by Rous.[4] It was therefore decided to subject the sterile and bacteria-laden tissues to the action of the disinfectants for a definite period before their incubation in tissue cultures. The tissues were cut into small

[1] Lambert, R. A., Technique of Cultivating Human Tissues *in Vitro*, *J. Exp. Med.*, 1916, xxiv, 367.

[2] I am indebted to the surgical staff of the hospital for their cooperation in supplying me with fresh tissue.

[3] Dakin, H. D., On the Use of Certain Antiseptic Substances in the Treatment of Infected Wounds, *Brit. Med. J.*, 1915, ii, 318.

[4] Rous, P., The Growth of Tissue in Acid Media, *J. Exp. Med.*, 1913, xviii, 183.

bits of a size suitable for culture preparations (about 0.5 mm. in diameter). These were washed quickly in one change of isotonic salt solution to remove the excess of blood and serum, and were then transferred to a dish containing a 24 hour broth culture of *Staphylococcus aureus* diluted five times with isotonic salt solution. The tissue fragments were allowed to soak for several minutes so as to become permeated by the bacteria. They were then transferred to dishes containing varying strengths of the chemicals to be tested. In these they remained for exactly 1 hour and were then removed to a dish of isotonic salt solution until put up in culture preparations,—usually a few minutes to half an hour. A second series was made with sterile tissues. Two sets of controls were prepared; one of untreated non-infected tissue and one of infected tissue. The tissue cultures were prepared as described in a previous paper,[1] using as a culture medium chick plasma and human serum in the proportions of 1 to 4. Five preparations were made for each strength of disinfectant used and for each of the controls. Besides the antiseptics in common use several other chemicals were tested, and the results are included in Table I. Many preliminary tests were necessary to approximate the lethal strengths of the chemicals for bacteria and cells. Since each experiment was repeated at least once the preparation of about 2,000 cultures was required.

DISCUSSION.

The table shows that in the case of the majority of the chemicals used (potassium cyanide, phenol, tricresol, hydrogen dioxide, and alcohol) tissue cells were definitely more easily killed than were bacteria. With certain other disinfectants the difference was not so striking. For example, in several experiments with mercuric chloride it was noted that in a few preparations there was a slight growth of connective tissue cells after exposure for 1 hour to a dilution of 1:20,000 or 1:40,000, a strength sufficient to kill or markedly inhibit the growth of staphylococci under similar conditions. It was observed, however, that the cells grew out from the centers of the tissue fragments, not appearing until after 4 to 5 days of incubation. It was concluded that growth in these cases was due simply to the

TABLE I.

Dilutions.	Mercuric chloride.		Potassium mercuric iodide.		Potassium cyanide.		Hypochlorites (Dakin's solution).		Iodine.		Phenol.		Tricresol.		Argyrol.		Hydrogen peroxide.		Glycerol.		Alcohol.	
	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.	Cells.	Bacteria.
1 : 1																					0	0
1 : 5																			0	+	0	+
1 : 10																			±	+	+	+
1 : 20																			+	+		
1 : 50																	0	0				
1 : 100											0	0	0	0	0	0	0	±				
1 : 200											0	+	0	±	±	+	0	+				
1 : 500											+	+	0	+	+	+	±	+				
1 : 1,000							0	0	0	0	+	+	0	+								
1 : 1,500					0	+	0	±	±	0												
1 : 2,000					0	+	0	±	+	0±												
1 : 2,500			0	0	0	+			+	±												
1 : 5,000			0	±	0	+	±	+	+	+												
1 : 10,000	0	0	±	±	+	+	+	+														
1 : 20,000	0	±	+	+																		
1 : 40,000	±	±																				
1 : 80,000	±	+																				
1 : 120,000	+	+																				

0 = no growth; + = growth; ± = slight growth.

low penetrating power of mercuric chloride, for cultures in 1:80,000 never showed an active outgrowth of peripheral cells.

Alcohol in the strengths used (5, 10, 20, and 50 per cent) was found to be bactericidal in only the highest strength. On the other hand, it is noteworthy that human cells show no ill effects from exposure to 5 or 10 per cent alcohol for 1 hour. In one series noted there was indeed a better growth of the alcohol-treated tissues than of the controls. Further experiments, however, failed to demonstrate any definite stimulating action on the part of alcohol. The harmful effect of 20 per cent glycerol is probably referable to the partial desiccation of the tissues produced.

Iodine stands out as the one chemical tested to which cells were found to be more resistant than were staphylococci. A good growth of cells was seen after exposure to a 1:2,000 solution of iodine for 1 hour, a strength sufficient to sterilize the tissue completely in most instances.

These experiments afford further experimental evidence of the value of iodine as an antiseptic, and indicate that, at least in weak aqueous solution, it should not, as is often stated, injure or irritate the tissues.

It was observed, however, that iodine has the power of rapidly dissolving fibrin, a property, which, theoretically, should not be conducive to wound healing. A similar action by hypochlorites (Dakin's solution) was also noted. Although the wound-cleansing property of the latter, which evidently depends on this fibrin-dissolving function, is favorably emphasized by Dakin,[3] it would seem that the plastering together of wound surfaces by fibrin, which is thought to facilitate healing, would be prevented by the use on wounds of either iodine or the hypochlorites.

CONCLUSIONS.

The comparative resistance of bacteria and human tissue cells to antiseptics and other chemicals may be easily tested by tissue cultures under conditions which approximate those found in the living body.

A comparative study shows that while human cells (connective tissue and wandering cells) are highly resistant to many antiseptics,

they are in general more easily killed than bacteria (*Staphylococcus aureus*).

Of the antiseptics tested, which include mercuric chloride, iodine, potassium mercuric iodide, phenol, tricresol, hydrogen peroxide, hypochlorites (Dakin's solution), argyrol, and alcohol, the one which approaches most closely the ideal disinfectant is iodine, which kills bacteria in strengths that do not seriously injure connective tissue cells or wandering cells.

CHANGES IN PIGMENT EPITHELIUM CELLS AND IRIS PIGMENT CELLS OF RANA PIPIENS INDUCED BY CHANGES IN ENVIRONMENTAL CONDITIONS.

By EDUARD UHLENHUTH, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 46 to 48.

(Received for publication, June 10, 1916.)

One of the most important questions in the field of cell evolution is whether the fate of the cell is definitely determined as soon as it makes its appearance as an individual cell among the mass of embryonic cells of the developing body or organ, or whether the final type into which a cell will develop is determined by its environmental conditions, such as external physical and chemical factors. If the latter principle prevails during the process of the production of the organic form, the application of new external factors would create new types of cells not existing under ordinary natural conditions.

From recent experiments it has become evident that structures which we thought characteristic of certain species of cells are in no way specific for these cells but may also be assumed by other species of cells when brought under similar conditions. The above mentioned experiments may be divided into two groups, the first group comprising experiments in which an animal body served as the substratum for the development of the cells, while in the second group of experiments an artificial substratum was used.

The experiments of the first group have established three facts: (*a*) Embryonic cells of the same type may develop into different types as a result of their developing in different environments. Thus, primitive blood cells may develop into erythrocytes, if they become enclosed by the walls of blood vessels, or they may become granulated white blood cells, if they continue to develop outside the ves-

sels (1). More or less definite evidence was also obtained to show that certain relations exist between external factors and the various cell types into which the lymphoid hemocytoblasts outside the walls of the embryonic vessels finally develop (2). (*b*) Fully developed cells may undergo dedifferentiation into the embryonic state and then, if subjected to other conditions, may differentiate into another fully developed type of structure entirely different from the original. In the Nemertine *Lineus lacteus* Rathke, fully developed connective tissue cells (parenchymal cells) of small fragments including no part of the intestine, may dedifferentiate into a more primitive type of mesenchymal cell, the so called *Wanderzellen*, and then redifferentiate into the epithelial cells of the newly formed intestine (3). This circumstance also proves that even general structures characterizing large groups of cells, such as epithelium and connective tissue, are interchangeable and that a member of one group may be converted into an element of another group. (*c*) Highly differentiated cells may be directly converted into other highly specified cell types without dedifferentiation into the embryonic stage. This is true of the iris pigment cells which furnish the material for the regenerating lens after the loss of their pigment, after which they represent ordinary fully differentiated but unpigmented iris cells.

Through the discovery of tissue culture we have, so to speak, created a new type of body in which to grow a cell; *i.e.*, a new form of cell environment, in many respects different from the normal body that nature has given the cell in which to develop. None of the results of this group of experiments favor the view that specificity and constancy are fundamental features of cell evolution, for all cells, when compelled to grow within this new form of environment develop into cell types which, on the one hand are different from those existing in the animal body and, on the other hand, are characterized by a number of common structures, which designate them as tissue culture types. Champy (4), for instance, observed that the cells of four different organs—the kidney, the submaxillary, the parotid, and the thyroid of the rabbit—when cultivated outside the organism, change into one cell type; not only do the epithelium and connective tissue cells of one of these organs become indistinguishable from each other, but all the cells of all four organs become so much alike that it is no

longer possible to determine from which of these organs a given cell is derived. By applying different physical factors inside the substratum, such as a varying consistency of the medium and supporting surfaces, it has been proved that fundamental and general characteristics, typical of large groups of cells, such as the cuboid or cylindrical shape of the epithelium cells and the spindle shape of the connective tissue cells, become interchangeable.

From these facts it has been learned that we actually know very little about the developmental abilities of the body cells and the potentialities for differentiation they would exhibit if compelled to live under conditions different from those usually present within the organism. These facts more and more emphasized the part played by the external factors in cell development and led to the abandonment of the theory of cell specificity. Furthermore, it has become necessary to admit that our histological system, based upon certain characteristics which are assumed by the cell under conditions during the study of which this system was elaborated, has no more value than that of an artificial nomenclature, applicable to the organism only so long as it is studied under these particular conditions, and useful only as a means of gross orientation in the organism under the ordinary conditions. Through the relatively high constancy of these conditions in nature, we have been misled to believe that the types of our artificial system constitute the types of differentiation into which a certain cell must always develop, and that these cells develop into such types only because of a certain inherent structure which is present from the outset of the embryonic life of each cell; and, furthermore, that this structure, attached to the cell and unchangeable throughout its life, determines by its specificity the course of differentiation which characterizes this cell according to our artificial system of classification. As it has already been shown that many of the features thought to be specific can be changed by external factors, and that some of these changes are obviously operated and controlled by the laws of non-living matter, the question becomes more and more important as to what is produced in each cell by external factors, and what is due to an elementary structure —elementary in the sense that with its removal the cell would lose its attribute of cell.

As I (5) have been dealing with these problems in several recent papers I wish to present in the following short report another contribution to this subject and to describe some changes effected in explanted pigment epithelium cells and iris pigment cells, which I had the opportunity of observing during some work on these cells.

Material and Methods.

Fragments of iris and pigment epithelium from the adult leopard frog (*Rana pipiens*) were placed in hanging drops of a medium consisting, in a few instances, of two drops of plasma and one drop of muscle extract, but in most cases of two drops of plasma and one drop of aqueous humor. All the media were derived from the same species. The last described medium proved in many respects to be preferable to the first mentioned. In the first place it takes a long time to prepare the muscle extract, which in spite of all possible precautions sometimes becomes contaminated, whereas the aqueous humor (6), even when prepared in large quantities, requires only a short time and necessitates practically no special precautions in order to furnish a perfectly sterile medium. And, secondly, the coagulation effected by the plasma, when mixed with the aqueous humor, is much more satisfactory than the clotting of the ordinary muscle extract mixture and may sometimes last for several days.

EXPERIMENTAL.

Cells of iris and pigment epithelium when kept in the medium described above do not migrate as readily as do the cells of skin epithelium under the same conditions. Many fragments remained without signs of cell movement while under observation, that is to say, for several weeks, after which time they underwent degeneration. In most fragments from which cell migration took place we did not observe that the cells wandered out into the medium until from 2 to 6 days after explantation, while in cultures of skin epithelium this phenomenon was often visible as early as the night following explantation. The cells migrating into the medium are seldom as numerous in cultures of iris and pigment epithelium as in cultures of skin epithelium. Furthermore, we have not thus far been able to de-

tect any mitosis in the iris pigment cells and pigment epithelium cells when explanted.

Both types of cells are able to form membranes around the original fragments, the pigment epithelium cells producing membranes which resemble a typical epithelium more closely than the membranes formed from iris pigment cells. The membranes which are formed around fragments of iris are sometimes composed of iris pigment cells, unpigmented iris cells, and connective tissue cells (Fig. 1). In these membranes the unpigmented iris cells may be indistinguishable from the connective tissue cells.

As a rule, however, the iris pigment cells, as well as the pigment epithelium cells, leave the fragments without being grouped into membranes. They spread out from the edges of the fragment as single cells, or form very thin and delicate and sometimes branching chains of cells, or narrow processes ending in a very fine fimbriation formed by the elongation of each terminal cell into the medium. The last two forms are typical of the iris pigment cells.

Before reporting on the aspect which these cells assume when migrating into the medium, I wish to call attention to some of the chief features of the pigment epithelium cells and iris pigment cells under ordinary conditions.

The structures of the normal pigment epithelium cells are particularly characteristic, as many of them are not found in any other cells of the animal body. There are six characteristics which should be kept in mind when comparing these cells with the migrating pigment epithelium cells. (*a*) The cell body is differentiated into two distinct parts, one of which faces the choroid, with which it is in close contact. Its more or less homogeneous protoplasm contains the cell nucleus as well as several probably metaplasmatic structures, among which the yellow oil globules are present in almost every cell, and, as a rule, can also be seen in the migrating cells (Fig. 2). The other part of the cell, the so called pigment basis, faces the retina and consists mainly of a dense mass of rod-shaped, yellowish brown pigment granules. (*b*) These pigment granules are arranged irregularly, with no common direction of their long axis. (*c*) From the pigment basis many chains of pigment granules, the so called pigment strains numbering, according to Angelucci (7), up to 40, originate and radiate

toward the retina. As far as has been ascertained it appears that these chains are protoplasmatic processes containing the pigment granules. (*d*) The protoplasmatic part of the cell is partly covered by a firm cuticular cap, by means of which, as well as by means of a number of cuticular rods of fine arrow-like shape, the cells are fastened together. (*e*) Seen from the basal surface the pigment epithelium cells appear to be of hexagonal shape. (*f*) It has never been proved that these cells are capable of any kind of movement within the organism, either under normal or pathological conditions and in the numerous eyes which I have examined at different stages of development, degeneration, and regeneration I have never been able to observe the slightest sign of movement on the part of these cells.

As far as this latter quality is concerned, the normal iris pigment cells entirely resemble the pigment epithelium cells, but in all other respects fundamental differences exist between these two species of cells. The iris pigment cells are not differentiated into two parts, but the spherical pigment granules with which the cell body is charged are distributed evenly throughout the whole cell. Pigment strains are lacking, all the cell boundaries being uniformly straight and unbroken. A simple basal membrane covers the basal surface instead of a highly differentiated cuticular cap. The shape of these cells is cuboid.

These same cells when migrating into the medium present a different appearance; the changes in the pigment epithelium cells are particularly conspicuous.

Figs. 2 and 3 show two pigment epithelium cells which have migrated into the medium and placed themselves at the edge of a membrane formed by these cells around the tissue fragments. The culture did not show cell migration until the 6th day. The cells shown in Figs. 2 and 3 were drawn on the 10th day after explantation, while in the act of isolating themselves. None of the pigment epithelium cells which are wandering out into the medium show the differentiation into a plasmatic part and a pigment basis which is so typical of the normal type of these cells. On the contrary, the cell body as shown in Figs. 2 and 3 is of the same structure throughout, the pigment being evenly distributed in the protoplasm. As the pigment granules are moved around slowly but continuously by the slow and

wave-like movements of the cell plasma they sometimes congregate more densely at certain points or may even occasionally be entirely shifted from one spot, leaving the cytoplasm free of pigment for a space of several minutes. The pigment granules no longer appear as an irregularly arranged mass, but most of them are now situated so that their long axis runs parallel to the long axis of the cell and to the direction of the cell medium. The most striking change is the complete disappearance of all the pigment strains. The cell border is smooth, and fringe-like protuberances are no longer visible.

Throughout all these changes the pigment epithelium cells have, in their appearance, approached much more closely the structure of a normal iris pigment cell. They no longer present typical differentiation into two parts and they have lost the pigment strains. This resemblance becomes still more marked if we compare the migrating pigment epithelium cells with the migrating iris pigment cells (8) instead of with normal ones, because the migrating iris pigment cells show the same slow movements of the pigment granules, by means of which several parts of the cytoplasm may become free of pigment for a short time.

From the changes observed thus far, it is evident that the cultivated pigment epithelium cells have lost all the features which characterize them in the organism as a result of the position which they there occupy between two essentially different elements, the retina and the choroid. In no cell hitherto examined in tissue cultures has the different action upon cell structure of various external factors been so conclusively demonstrated as in the pigment epithelium cells of the eye. While the effect of these various factors can be ascertained from an examination of the normal epithelium cells, the result of uniform environment is seen in the cultivated cell. As long as the cell is situated between two different media, each side shows a different structure; but as soon as it is surrounded by a uniform medium, a uniform structure must develop on all sides of the cell.

Besides the changes mentioned, other important differences between the cultivated and normal cells are visible. Both the pigment epithelium cells and the iris pigment cells lose all their individual structures. This is particularly conspicuous in the case of the former, because the cuticular cap of these cells is much more highly differ-

entiated than the simple basal membrane of the iris pigment cells and it produces a firm texture. The cuticular cap, as well as the basal membrane, has disappeared and thus both cells have become alike in that they lack cuticular structures. But in order to assume this character the pigment epithelium cell had to give up much more than the iris pigment cell, and this relation holds good for all other changes. Although this characteristic, *i.e.*, the lack of cuticular structure, is a feature common to all cultivated cells, it is particularly interesting in the present case, because the relation between the disappearance of this structure and the existence of uniform environment is most distinctly marked with regard to the pigment epithelium cell.

Finally we arrive at the shape and movement of both these cell types. Although they obey the ordinary rules which affect all cultivated cells and show, like the latter, a close relation between both phenomena, it appears to me of special significance that these cells behave in this respect like all other cells thus far examined in the tissue culture condition, because (*a*) a movement of these cells has never been observed within the body, while movements of most other cultivated cells, such as connective tissue, endothelial, and epithelial cells, commonly occur within the organs; and (*b*) the shape of the cultivated pigment cells is extraordinarily different from the original shape; in order to approach the usual shape of the free moving tissue culture cells, these cells must undergo enormous changes. This is shown in Figs. 4 to 10, which are drawn from cells moving in a semifirm (9) medium close to the cover-slip but without being attached thereto. Figs. 4, 5, and 6 show a single pigment epithelium cell, while Figs. 7 to 10 represent iris pigment cells. It will be seen that both cell types closely resemble each other also when in the condition of free movement. They are both spindle-shaped, like connective tissue cells, and show a similar disposal of the pigment. As a rule, the pigment is more dense in that part of the cell which lies backward of the direction of movement, than in the part flowing forward into the medium. In comparing Fig. 10 with a normal iris pigment cell the marked difference in shape is apparent.

In Figs. 4 to 6 the movement of one cell has been followed; this is seen to bear a close resemblance to the mode of movement of the epi-

thelium cells of the skin as described in a former paper (10). Fig. 4 was drawn at 3.30 p.m. while a long process at the rear was retracted into the cell and the pigment was accumulated into a dense pigment mass behind the nucleus. At 3.40 p.m. two processes at the front, which had already commenced to form at 3.30 p.m., were seen to be flowing out into two long pseudopodia, the space between which at 5 p.m. became more and more filled with the cytoplasm uniting both pseudopodia into one single process, the end of which was dichotomically branched (Fig. 6). The cell at the same time became narrower as a result of being elongated.

Although some important individual characteristics still persist in both cell types, nevertheless the changes already effected undoubtedly prove the importance of external factors in producing cell structures. Furthermore, it may be mentioned that prolonged cultivation under more favorable conditions may even change those structures which could not be influenced during the short time employed in our experiments. Attention should be called to the fact that all the changes described were established directly in the original cell and not gradually in cells derived from it by mitosis, for cell division was never observed to occur in these cultures.

The experiments seem to indicate that certain important and essential differences must exist between the retina and choroid in order to produce such peculiar structural differences of two sides of one cell. Further experiments may serve to throw some light upon the nature of these differences.

SUMMARY.

1. Retina pigment epithelium cells and iris pigment cells are placed in an identical medium.

2. The differentiation into two different parts, as well as the pigment strains of the migrating pigment epithelium cells, is lost. The normally highly specialized cuticular structures disappear. The cells begin to move and assume a spindle shape, thus losing their original hexagonal shape.

3. The migrating iris pigment cells begin to show a distribution of the pigment, similar to that exhibited by the migrating pigment

epithelium cells. The cuticular structures of this cell type also disappear. These cells also assume the ability of free movement and become spindle-shaped.

4. By means of these changes both cell types not only become more similar to the ordinary culture cell type, but also in certain respects, more like each other, whereas before explantation they were very unlike. In order to accomplish this the pigment epithelium cells must change much more thoroughly than the iris pigment cells.

5. The changes, particularly those of the pigment epithelium cells, show a definite relation to the changes of external factors, as they are the expression of the transmission from dissimilar conditions existing on different sides of the cells into conditions which are uniform on all sides.

In conclusion I wish to acknowledge the assistance of Mr. Nelson Anderson, who greatly facilitated this work.

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10. Uhlenhuth, The Form of the Epithelium Cells in Cultures of Frog Skin, and Its Relation to the Consistency of the Medium, *J. Exp. Med.*, 1915, xxii, 86 and following pages, and Figs. 9 and 12.

EXPLANATION OF PLATES.

Subtract 18 mm. from the length of the tube given, in order to obtain its actual length. All the figures have been drawn at the level of the table instead of at the level of the microscope desk. The pictures are therefore on an average 32 per cent (linear) larger than when seen by the magnification of the lens system alone.

PLATE 46.

FIG. 1. Iris and connective tissue cells forming a membrane around a fragment of the ventral part of the iris of *Rana pipiens*, which had been placed 4 days previously in two drops of plasma plus one drop of muscle extract of the same species. Drawn from the living specimen (Culture E 85), with the aid of an Abbé camera lucida. Leitz oc. 3; obj. 3.

PLATE 47.

FIGS. 2 and 3. Pigment epithelium cells of *Rana pipiens* migrating into the medium, drawn from the living specimen (Culture E 303), 10 days after the original fragment had been placed in two drops of plasma and one drop of aqueous humor of the same species. Abbé camera lucida; Leitz oc. 3; obj. $\frac{1}{12}$; oil immersion; tube 130.

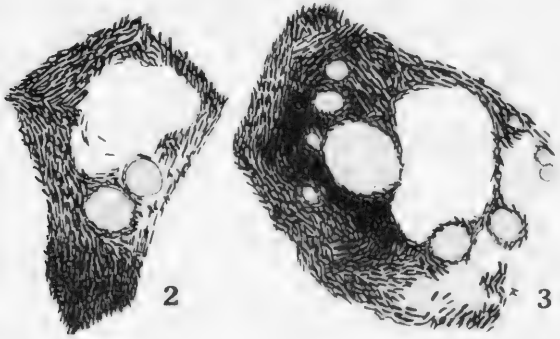
FIGS. 4, 5, and 6. An individual pigment epithelium cell of *Rana pipiens*, moving in two drops of plasma plus one drop of muscle extract (Fig. 4 at 3.30 p.m., Fig. 5 at 3.40 p.m., and Fig. 6 at 5 p.m.). Culture E 86. Abbé camera lucida; Leitz oc. 3; obj. $\frac{1}{12}$; oil immersion.

PLATE 48.

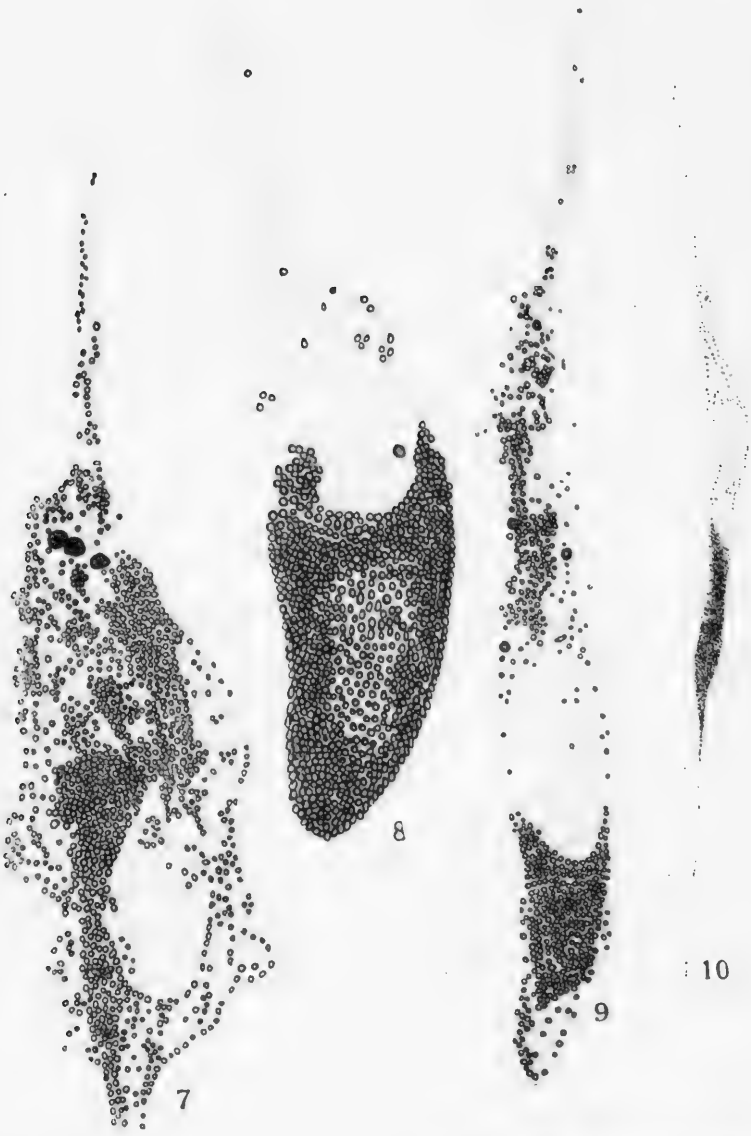
FIGS. 7, 8, 9, and 10. Four moving iris pigment cells in a semifirm medium. The original fragment (Culture E 276), a small piece of the dorsal part of the eyes, did not show cell movement until the 5th day. Figs. 7 and 8 were drawn on the 11th day after explantation; Figs. 9 and 10 on the 12th day after explantation. Abbé camera lucida; magnification of Figs 7, 8, and 9, Leitz oc. 3; obj. $\frac{1}{12}$; oil immersion; tube 130. Magnification of Fig. 10, Leitz oc. 3; obj. 6; tube 130.

1

(Uhlenhuth: Changes in Cells of *Rana pipiens*.)



(Uhlenhuth: Changes in Cells of *Rana pipiens*.)



(Uhlenhuth: Changes in Cells of *Rana pipiens*.)

THE SEPARATION OF SERUM INTO COAGULATIVE AND NON-COAGULATIVE FRACTIONS.

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It is known that diphtheria antitoxin is associated in horse serum with its pseudoglobulin constituent. For therapeutic purposes this protein fraction has been extracted from the whole serum, thus obtaining a purer, or refined antitoxin preparation. It seemed possible that the same principle might be applied to the coagulative factor in serum, and that in this respect the active substance might likewise be linked with one protein fraction rather than with the serum as a whole. Horse serum is extensively used for hemostatic purposes, and if the coagulative principle could be separated, it might lead to the preparation of a refined hemostatic as potent as the original serum and containing a greatly diminished amount of protein. With this end in view, the albumin, the pseudoglobulin, and the euglobulin were separated by means of ammonium sulphate of various strengths and were tested for their coagulative efficiency. A brief report of this work was made some months ago.[1] Although this process does not yield protein fractions of absolute purity, it is the best method for the purpose, affording a sharp demarcation between the group of albumins and globulins, and a fairly sharp division between the soluble pseudoglobulin and the less soluble euglobulin.

The method followed was the one used in this laboratory for the preparation of refined diphtheria antitoxin. It consists of diluting the serum with one-half of its volume of water, and then precipitating the euglobulin with a 30 per cent ammonium sulphate solution; a small amount of pseudoglobulin comes down in the course of the process. The ammonium salt is then added up to 54 per cent to carry down

[1] Hess, A. F., *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 125.

the pseudoglobulin, after which enough is added to the filtrate to precipitate all the albumin.[2]

TABLE I.

The Coagulative Effect of Adding the Protein Fractions of Human Serum to Normal Human Blood.

Control blood.	Euglobulin.		Albumin.		Pseudoglobulin.		Time.
	1 drop.	2 drops.	1 drop.	2 drops.	1 drop.	2 drops.	
							min.
−	−	−	−	−	−	−	2
+	+	+	+	+	+	+	4
++	++	++	++	++	++	++	8
++	+++	+++	++	++	++	++	10
++			++	++	++	++	12
+++			+++	++	++	+++	15
				+++	+++		18

Tests with Human Serum.

The nature of the tests and their results are shown in the tables. For the test illustrated in Table I, the fractions were prepared from human serum. It was determined in the course of later tests that the fractions had apparently identical action whether prepared from human, from horse, or from sheep serum; accordingly horse serum was used as it was most available. In view of the fact that it had been found that antitoxin is associated with the pseudoglobulin in the horse, but with the globulin in the serum of the goat, it was deemed advisable to carry out the preliminary tests upon human as well as upon animal sera.[3] It will be noted from Table I that the addition of one or two drops of euglobulin hastened coagulation, whereas the same amount of the other proteins failed to bring about this result. This was the usual course of all experiments of this kind, although in some instances the differences did not stand out so sharply.

[2] These separations were carried out by Mr. Edward Banzhof.

[3] Steinhardt, E., and Banzhof, E., unpublished work of the Research Laboratory of the Department of Health, New York.

TABLE II.

The Coagulative Effect of Adding the Protein Fractions of Human Serum to the Blood and Plasma of Hemophiliacs.

(*A*) *Case 1. Blood.*

Control blood.	Euglobulin.		Seroalbumin.		Pseudoglobulin.		Time.
	1 drop.	2 drops.	1 drop.	2 drops.	1 drop.	2 drops.	
							min.
−	−	−	−	−	−	−	4
−	+	+	−	−	−	+	8
++	++	+	−	−	+	+	12
++	++	++	+	+	+	+	14
++	++	++	++	++	++	++	18
++	+++	+++	++	++	++	++	20
++			++	++	++	++	24
++			++	++	++	++	28
++			+++	+++	++	++	34
++			+++		++	+++	36
+++					++		42

(*B*) *Case 2. Plasma.*

−	−	−	−	−	−	−	6
−	+	++	−	−	−	−	8
+	+	+++	+	+	+	+	10
+	++		+	+	++	+	12
+	+++		+	++	++	++	14
+			++	+++	++	++	16
++			+++		+++	+++	18
+++							26

Table II illustrates a similar test carried out in a case of hereditary hemophilia, where the blood had a coagulation time of 42 minutes and the plasma of 26 minutes.[4] Here again the euglobulin caused a marked shortening of the coagulation time, whereas the other fractions produced this effect to a less degree.

When it was not desired to obtain the three protein fractions separately but only to precipitate the euglobulin from the serum,

[4] For the coagulation tests of blood a simple method suggested by Howel (*Arch. Int. Med.*, 1914, xiii, 76) was employed. The basilic vein was punctured and 15 drops of blood were allowed to flow from the needle into a vial having a diameter of 1.5 cm. When the vial could be inverted without spilling any of its contents, coagulation was considered to be complete.

sodium chloride was employed instead of ammonium sulphate, as the former process is simpler and was found to give an equally effective product. The method pursued was as follows: The serum was saturated in the cold with chemically pure crystals of sodium chloride. It was then filtered, and a small amount of chloroform added to the precipitate, which was dialyzed for about a week. The dialysate was taken up with Ringer's solution, and 0.3 per cent of tricresol added. This was then filtered through a Berkefeld filter and bottled in 20 cc. vials.

The percentage of total solids, which may be regarded as euglobulin, varied in the different lots prepared, so that an estimation was made in every instance. In the lot used in the test shown in Table III, there was 2.5 per cent euglobulin, in another the percentage might

TABLE III.

The Action of Different Concentrations of Euglobulin on Purpuric and on Normal Plasma.

Purpuric Plasma.

Control plasma.	Euglobulin.		Time.
	1.25 per cent, 1 drop.	2.5 per cent, 1 drop.	
			min
−	−	−	2
+	+	+	4
+	++	++	8
++	+++	+++	10
++			14
++			18
++			22
+++			26

Normal Plasma.

Control plasma.	Euglobulin.				Time.
	1.25 per cent.		2.5 per cent.		
	1 drop.	2 drops.	1 drop.	2 drops.	
					min.
+	+	++	++	++	4
+	+++	+++	++	+++	6
++			+++		8
+++					10

be over 3 per cent, in which event it was diluted with Ringer's solution to 2 per cent. This test (Table III) shows that when we reach a certain maximum, the addition of a large amount of euglobulin ceases to have any further effect; that, for example, the coagulation time may remain the same whether we add a drop of a 1.25 per cent solution or of a 2.5 per cent solution of euglobulin to five drops of plasma. In another test (Table IV) made with a different preparation we note that two drops are more effective than one, but that we have then reached the optimal strength, and that three drops of

TABLE IV.

The Comparative Activity of Whole Serum and Euglobulin on Human Plasma.

Control plasma.	Whole serum.			Euglobulin, 2 per cent.			Time.
	1 drop.	2 drops.	3 drops.	1 drop.	2 drops.	3 drops.	
							min.
−	−	−	−	−	+	+	4
−	−	+	+	−	+	+	6
+	+	+	+	−	++	+	8
+	+	+	+	+	++	++	10
+	+	+	+	+	+++	+++	12
+	+	++	+	++			16
++	+	+++	+++	+++			20
+++	+++						22

euglobulin fail to heighten the effect. Table III likewise illustrates the fact that the euglobulin is able to hasten the clotting of plasma in purpura, another type of hemorrhagic disease. In this instance the blood was from a case of purpura showing all the usual symptoms—a slightly delayed coagulation time, a markedly diminished platelet count, a prolonged bleeding time, and a positive capillary resistance test.

Mode of Action of Euglobulin.

There are many ways in which substances exert a coagulative effect upon the blood. Without entering in detail upon this subject, it may be stated that we may divide these acceleratory substances into two groups; those which act by neutralizing inhibiting substances, and those which possess inherent activity in themselves.

To which class does euglobulin belong? The answer to this question can best be given by comparing the euglobulin to a substance which is known to derive its potency through overcoming the antithrombin of the blood. An experiment of this kind is reproduced in Table V, where the effect of tissue juice is compared with that of euglobulin.

TABLE V.

Comparison between the Neutralizing Power of Euglobulin and of a Thromboplastic Solution when Hirudin Was Added to Plasma.

(*A*) *Titration of Euglobulin and Thromboplastin Solution to Equal Strengths.*

Control plasma.	Euglobulin.		Thromboplastin, 1 drop.			Time
	1 drop.	2 drops.	1 : 1,250	1 : 2,500	1 : 5,000	
						min
+	+	+++	++	++	+	2
++	+++		+++	+++	++	3
++					+++	4
+++						6

(*B*) *Relative Coagulative Potency of the Solutions when Hirudin Was Added.*

Plasma + hirudin.*	Euglobulin, 1 drop + hirudin.	Thromboplastin, 1 : 2,500 + hirudin.	Time.
			min.
−	−	++	4
+	+	+++	6
+	+		8
+	+		10
+	++		35
+	+++		42
++			120
+++			130

* Hirudin in these tests refers to a 1 : 5,000 solution. This strength was found optimal for this experiment.

It has long been known that the various tissue juices of the body, those of the muscles, of the brain, of the glandular organs, etc., are powerful coagulants. It has also been established that they possess this power in virtue of being able to counteract the normal antithrombin of the blood. In this test thromboplastin was employed, which consists of a solution and suspension of brain, and has been

described elsewhere[5] as possessing marked coagulative power both *in vitro* and in its clinical application. In the first part of this table we see what might be termed a state of equilibrium between the two coagulants, a parallel test in which one drop of euglobulin and one drop of a dilution of thromboplastin (1: 2,500) hasten coagulation to the same degree. When, however, an antithrombic substance, a dilute solution of hirudin, is also added to the plasma, we note (Table V) a marked difference in action. The tissue juice almost completely neutralizes the hirudin, whereas the euglobulin is able only partly to overcome its inhibitory effect. It is evident therefore that these substances act in different ways, and that the euglobulin does not owe its power to a counteraction of the antithrombin. It is impossible to proceed further in this discussion, and to state the exact mode of action of euglobulin, as so many factors remain unknown as to the mechanism of coagulation. It would seem, however, in view of the fact that euglobulin is found in the normal blood, that it should be regarded as a physiologic coagulant, and its mode of action in these tests as merely an intensification of the normal clotting processes. In this connection we may add that this coagulative property is not common to globulins in general. For example, egg globulin showed no coagulative power (Table VI); and edestin, a vegetable

TABLE VI.

The Comparative Effect of Ovoglobulin and Ovoalbumin when Added to the Blood of a Hemophiliac.

Control blood.	Euglobulin. 1 drop.	Seroalbumin. 1 drop.	Ovoglobulin. 1 drop.	Ovoalbumin. 1 drop.	Time.
					min.
+	++	−	+	−	4
+	++	−	+	−	6
++	++	−	+	−	10
++	++	+	+	−	14
++	+++	+	+	−	16
++		+	+	+	18
++		+	++	++	20
+++		++	++	++	30
		+++	++	++	38
			+++	+++	52

[5] Hess, *J. Am. Med. Assn.*, 1915, lxiv, 1395.

globulin, when tested in various concentrations was found also to be completely devoid of activity. These negative results with ovoglobulin furthermore give evidence that the more rapid clotting, brought about by euglobulin, cannot be attributed to an increase in viscosity.

SUMMARY AND CONCLUSIONS.

The fact that the coagulative principle is closely associated with the euglobulin fraction of the blood is of clinical as well as of theoretical interest. It enables us to prepare a hemostatic containing about 2 per cent of protein which is more potent than the whole serum containing 6 to 7 per cent of protein. A preparation of this kind has been made in this laboratory from horse serum and employed during the past few months in numerous cases of bleeding. This euglobulin is absolutely sterile, as it has been passed through a Berkefeld filter, and is safeguarded against decomposition by the addition of 0.3 per cent tricresol. A detailed account of the therapeutic use of euglobulin will be reported elsewhere. It may be stated, however, that it has been employed in the various manifestations of intractable hemorrhage in which horse serum has been so largely resorted to of late years, and that in certain cases has seemed to bring about most satisfactory results; in no instance has there been any untoward effect. When intravenous injections are resorted to, euglobulin seems to be preferable to serum which contains fully three times the quantity of protein.[6] It also seems to be absorbed more quickly from the subcutaneous tissues. In all probability it will be found to meet the same indications as whole serum, possessing the advantages of concentration, and necessitating the introduction into the body of a much smaller amount of foreign protein.

[6] Refined diphtheria antitoxin is frequently used as a hemostatic when normal serum cannot be obtained. As this serum contains about 16 per cent of protein, which consists almost entirely of pseudoglobulin, it will readily be appreciated that it is not adapted to this use.

THE INFLUENCE OF CERTAIN FACTORS, ESPECIALLY EMOTIONAL DISTURBANCES, ON THE EPINEPHRIN CONTENT OF THE ADRENALS.*

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In a study, mainly in cats, of the epinephrin discharge caused by electrical stimulation of the splanchnic nerves[1] we were led to consider the question whether the amount of epinephrin liberated by a given strength and duration of stimulation is related to the store of epinephrin already present in the adrenals, or is independent of that store. Desiring to compare the amount liberated in animals whose content was presumably high with the amount liberated in animals whose content was presumably low, we tried to exhaust the store, as far as possible, before the experiment on the liberation was made, by some of the procedures which according to Elliott[2] cause diminution of the epinephrin load. Morphine was tried and also β-tetrahydronaphthylamine, since Elliott states that these drugs produce marked exhaustion. But as in cats they also cause wide dilatation of the pupil, and we were using the eye reactions as one of the criteria of the presence of epinephrin in the blood, we eventually had recourse to frightening the cat by a dog. Elliott attributes the loss of epinephrin from the glands, induced by morphine and β-tetrahydronaphthylamine, to the fright occasioned in cats by these substances. But he did not make any experiments on the effect of actual fright, although he says that "the most direct

* A preliminary note was published in *Proc. Soc. Exp. Biol. and Med.*, 1916, xiii, 184.

[1] Stewart, G. N., Rogoff, J. M., and Gibson, F. S., *J. Pharm. and Exp. Therap.*, 1916, viii, 205.

[2] Elliott, T. R., *J. Physiol.*, 1912, xliv, 374.

method of analysis would be that of inducing emotional fear at once, as by vexing the cat with a dog." He refers to the experiments of Cannon and de la Paz[3] as supplying evidence of that nature, since "they showed that the emotion of anger or fear is associated with the appearance of adrenalin in the blood from a cat's suprarenal vein." We do not see that an increased liberation of epinephrin into the blood would necessarily imply a diminution in the epinephrin store of the glands. Elliott himself has given an instance of epinephrin liberation into the blood without any change in the store; namely, the liberation caused by electrical stimulation of the splanchnics. We shall produce evidence in another section of the paper that splanchnic stimulation can induce exhaustion of the epinephrin store, although far less easily than morphine, etc. It is, however, certain that a considerable amount of epinephrin can be discharged into the blood as a result of electrical stimulation of the splanchnics without any appreciable diminution being produced in the store. Indeed, the epinephrin passes into the blood at a more rapid rate than when procedures which distinctly exhaust the store (ether anesthesia, morphine, etc.) are employed. The load of epinephrin present at any given time in an adrenal would seem merely to represent the balance between formation and excretion, its absolute amount giving no index of the rapidity with which the epinephrin is built up and given off. Accordingly, even if it is assumed that fright causes increased liberation of epinephrin into the blood, the question still remains open whether the stock in the glands is diminished.

As a matter of fact, we did not find that in frightened cats the amount of epinephrin which could be excreted into the blood in response to splanchnic stimulation was less than in animals in which the experiments were made in the usual way without preliminary frightening. Nor did the assay of the epinephrin in the adrenals at the end of the experiment indicate that the stock had been appreciably exhausted by the frightening. This induced us to make some observations on the influence of emotional disturbances on the epinephrin content. The content was assayed by the method of Folin,

[3] Cannon, W. B., and de la Paz, D., *Am. J. Physiol.*, 1911, xxviii, 64.

Cannon, and Denis.[4] They compared the colorimetric method with the blood pressure method in pithed cats as described by Elliott and state that the two methods gave the same results. We made comparisons in two experiments and found also a sufficiently close agreement. We used Ringer extracts of adrenals and also acid extracts prepared according to the directions of Folin, Cannon, and Denis.

The colorimetric method proved to be satisfactory for the comparative estimations with which we were alone concerned. Where only small differences in the depth of the tint of the extracts of the two glands were present, we always checked the determinations with the standard by an observation in which the extracts of the two glands were directly compared with each other in the Duboscq colorimeter. The determination was always completed within 3 minutes, at most, after addition of the sodium carbonate solution.

Elliott states that animals killed without section of the nerve supply of one adrenal show equality of load in the two glands. We have seen abundant confirmation of this statement, although occasionally a difference well beyond the limits of error of the epinephrin assay exists, possibly more frequently in dogs than in cats (Table I). A similar equality was found in animals dying spontaneously, either of disease or after operations (Tables XIII and XIV) when the nerves of both glands were intact.

TABLE I.

Species.	Weight of adrenal.		Epinephrin.		Remarks.
	Left.	Right.	Left.	Right.	
	gm.	*gm.*	*mg.*	*mg.*	
Dog....	0.355	0.354	0.51	0.51	Killed with amyl nitrite and ether.
"	1.485	1.720	1.64	1.80	" by bleeding from carotids. Thyroid operation 24 hrs. previously.
Cat....	0.238	0.231	0.30	0.26	Killed by chloroform.
"	0.241	0.227	0.30	0.30	Shot through head.

[4] Folin, O., Cannon, W. B., and Denis, W., *J. Biol. Chem.*, 1912–13, xiii, 477.

TABLE II.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Duration of fright.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*	*days*	*hrs.*
Cat 1	0.261	0.224	0.28	0.22	5	5
" 2	0.326	0.250	0.24	0.25	5	5½
" 3	0.225	0.197	0.20	0.16	6	6
" 4	0.170	0.176	0.20	0.18	14	5½
" 5	0.210	0.221	0.25	0.24	10	5
" 6	0.165	0.188	0.14	0.15	33	5
" 7	0.154	0.156	0.16	0.18	33	5
" 8	0.200	0.196	0.16	0.15	33	5
Dog 1*	0.450	0.480	0.67	0.67	8	5
" 2†	0.625	0.621	1.00	0.92	5	5

* No. 1 was a small dog.

† No. 2 was a large male dog. Wound infected superficially. Infection did not extend into the abdomen.

Influence of Emotions on the Epinephrin Store of the Adrenals.

In Table II the results of eight experiments on cats and two on dogs are given. In the cats all the fibers coming to the left semilunar ganglion, including the major and minor splanchnics, had been divided according to Elliott's method. All operations were, of course, performed under ether anesthesia. Control experiments with morphine and with β-tetra showed that we invariably produced with these drugs the differential action on the two adrenals described by Elliott. Confirmatory evidence of the correctness of our technique was afforded by the marked differential effect observed in a number of the cats which died from various causes (Table XIV). It can, accordingly, be assumed with confidence that the innervation of the left adrenal in the cats used for the experiments was eliminated sufficiently to show definite differential exhaustion of the right, had the emotional disturbance been really associated with exhaustion.

Also, the length of time between the operation and the emotion experiment was sufficient to allow equality of load to be reestablished after the postoperative depletion of the unprotected adrenal, as was shown by control observations. For example, Cat 9 was killed suddenly 4 days after section of the fibers coming

to the left semilunar ganglion. The left adrenal weighed 0.330 gm. and contained 0.37 mg. of epinephrin; the right weighed 0.350 gm. and contained 0.37 mg. Cat 10 was suddenly killed 33 days after section of the fibers coming to the left semilunar ganglion. The left adrenal weighed 0.186 gm. and contained 0.17 mg. of epinephrin; the right weighed 0.200 gm. and contained 0.17 mg. of epinephrin.

The animals were subjected to emotional excitation for 5 to 6 hours by the presence of barking dogs. For this, the cats were enclosed in small cages so constructed that it was impossible for the dog to inflict physical injury upon the cat or to come in contact with it. The stimulation of ordinary sensory nerves was thus excluded as a factor. One of the observers personally took charge of the experiment from beginning to end. Of course, the emotions included fright, anger, fear, etc., the cats showing fight from time to time. The usual signs of sympathetic stimulation, dilatation of the pupil, erection of the hairs of the back and tail, etc., were naturally strongly elicited.

In some experiments dogs previously prepared by interference with the innervation of one adrenal were used to frighten prepared cats, two experiments on emotional disturbance being thus done with the same trouble as one. Control experiments with morphine (Table III) showed that the operations selected permitted a good differential effect, and this was confirmed in the case of prepared dogs dead of infections (pneumonia) (Table XIV).

In Dog 2 (Table II) the left major and minor splanchnics were cut in the abdomen. In Dog 1 the same nerves were divided, but, in addition, nerve strands coming towards the left adrenal from the lumbar sympathetic chain were severed.

It was shown by experiments with morphine that in dogs division of the major and minor splanchnics suffices to give a differential effect. In some of our animals, however, to be certain that enough of the innervation had been eliminated, we excised in addition the two lumbar ganglia next below the diaphragm and cut any strands seen coming from the lumbar sympathetic chain. In two of the animals, three lumbar ganglia were removed, including one above the diaphragm, which was perforated for the purpose. Not all these animals were employed for the observations on emotion; but to save repetition the operations used also for the morphine experiments, to be discussed in the next section, are given here. Some of the morphine experiments are mentioned in this section, since they serve as controls to show that the operations relied on were effective.

It was found that section of the major splanchnic only was not sufficient to give differential protection against exhaustion of the epinephrin store under morphine (Dog 9, Table III). Individual variations, of course, possibly exist in different dogs in this regard. In a dog (No. 17, Table XIV) dead of pneumonia, 13 days after division of the left major splanchnic, the right gland removed after death was found to be exhausted relatively to the left (0.31 mg. of epinephrin as

compared with 0.50 mg.). In another dog (No. 18) also dead of pneumonia, 14 days after section of the left major and minor splanchnics, excision of the two lumbar ganglia immediately below the diaphragm, and section of the strands going to the semilunar ganglion, marked protection of the load of the left adrenal as compared with the right was found (0.30 mg. in the left, 0.08 mg. in the right).

There is no doubt then, that the operations practised by us would have sufficed to reveal a relative exhaustion of the epinephrin store in the gland with the intact nerve supply under the influence of emotions, had such emotions been capable of causing exhaustion.

It will be seen from Table II that neither in the cats nor in the dogs is there any clear and constant deficiency in the epinephrin load of the right (still innervated) adrenal as compared with the left (denervated) gland. A difference of the same order of magnitude as that in Cat 1 may occasionally be seen in cats suddenly killed without operation (Table I). Our experiments yield no evidence that under emotional stress epinephrin is poured out into the blood in such quantities as to produce a decided impression upon the epinephrin store. In observations on the spontaneous liberation of epinephrin under experimental conditions,[5] we were struck by the steadiness rather than by the mobility of the rate of discharge. Attempts to produce acute changes in the rate in various ways were always without success. We were never fortunate enough to test adrenal vein blood at a moment when an outburst was taking place.

Whether experiments on the epinephrin content of the adrenals under the influence of emotional disturbance continued for a much longer time would yield a different result, we have, of course, no evidence. If the rate at which epinephrin passes into the blood were increased by fright it is conceivable that the rate at which it is built up, accelerated at first to keep the balance even, would eventually decline, thus permitting a deficiency in the load to be established.

Is the Morphine Depletion in Cats Due to Fright?—If emotion *per se* does not cause exhaustion of the epinephrin store the question at once arises whether the morphine effect in cats ought to be attributed to associated fright or to some other action of the drug. An easy way of testing this was afforded by the well known difference

[5] Stewart, G. N., and Rogoff, J. M., *J. Pharm. and Exp. Therap.*, 1916, viii, 479.

in the action of morphine upon cats and dogs. In dogs no signs of fright are, of course, produced. What happens, then, to the epinephrin load of the unprotected adrenal in dogs, as compared with that of the denervated gland, when morphine is administered? Elliott's result on morphinized cats is easily confirmed, but it is scarcely more difficult to prove that the same qualitative result is obtained on dogs. Accordingly we do not think that fright has anything to do with the morphine effect in cats.

TABLE III.

Morphine Effect on Epinephrin Store in Dogs.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Morphine.	Duration of morphine action.	Nerves cut.
	Left.	Right.	Left.	Right.				
	gm.	*gm.*	*mg.*	*mg.*	*days*	*mg.*	*hrs.*	
3	0.468	0.506	0.83	0.67	3	125*	7	Major and minor splanchnics.
4	0.430	0.384	0.67	0.37	4	125	8½	" " " "
5	0.280	0.284	0.36	0.23	10	150	8	" " " "
6	0.372	0.420	0.42	0.30	15	75	8	" " " " and 2 lumbar ganglia and chain excised below diaphragm.
7	0.550	0.565	0.30	0.21	11	80	8	Major and minor splanchnics, 2 ganglia below, and 1 above diaphragm.
8	0.711	0.754	0.97	0.98		150	7½	Control. No operation.
9	0.435	0.466	0.50	0.50	10	150	8	Major splanchnic only.
10	0.346	0.380	0.33	0.27	12	150	8	Only major splanchnic certainly cut.

* Morphine was given in two doses in all the animals, except No. 3, in which it was given in one dose.

Table III illustrates the results obtained with morphine in dogs. Remembering that section of the major splanchnic alone does not protect, or at least not constantly (Dogs 6 and 7), the reader will see that there is uniformly a definite deficiency of epinephrin in the right (innervated) gland as compared with the left (denervated) gland. A control animal (No. 8) morphinized for 7½ hours (without operation) had an equal load in the two adrenals.

TABLE IV.

Control Dogs Suddenly Killed.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Nerves cut.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
11	0.640	0.627	1.50	1.20	20 hrs.	Major and minor splanchnics.
12	0.400	0.408	0.72	0.72	9 days.	" " " "
13	0.640	0.550	0.83	0.83	4 "	" " " "

In Table IV results are shown on control dogs, with major and minor splanchnics cut, which were killed without being subjected to the action of morphine. The load in the two adrenals is seen to be equal, except in Dog 11, which was killed 20 hours after the operation. In this animal, as will be shown in the section on the postoperative deficit, the deficiency in the right (unprotected) adrenal no doubt represents merely the deficiency invariably seen after an operation and which has not been recouped in the short interval of 20 hours.

That signs which might be interpreted as those of fright are present in cats under morphine is, of course, not doubtful. Whether this interpretation is correct might be difficult to decide, and does not concern us here. It is, however, of interest to note that epinephrin seems to have nothing to do with those signs.

The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the splanchnic supply of the other cut and in which accordingly no liberation of epinephrin through the splanchnics takes place. A cat in this condition behaves identically in the same way as a cat whose adrenal splanchnic supply has been cut on one side but left intact on the other. The pupils are widely dilated and there is the same characteristic restlessness and incessant movement. The content of epinephrin in the remaining adrenal of the first cat is found to be practically the same as that of the adrenal removed before the administration of morphine, while the content of the adrenal with intact splanchnic supply in the second cat is definitely diminished.

The dilatation of the pupil of the denervated eye, and the pilomotor effects associated with fright and anger were also observed

in cats after removal of one adrenal (right) and section of the nerves of the other, when the animals were frightened by a dog and in other ways.

Observations were made on four cats (Table V) in this way.

TABLE V.

Cats with the Right Adrenal Removed and Nerves of the Left Adrenal Cut.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*	*days*	
11	0.316	0.302	0.25	0.30	1	Died.
12	0.280	0.280	0.19	0.28	9	"
13	0.270	0.250	0.28	0.14	3	Killed by ether.
14	0.220	0.168	0.08*	0.18	37	" after experiments.

* The left adrenal was massaged before removal, in the course of an experiment.

They all yielded the same results. Sudden fright, as by hitting or jerking the holder, caused dilatation of both pupils instantaneously; that is, after an interval too short to be measured with a stop-watch and certainly far shorter than the interval required when reactions are evoked in a denervated eye by epinephrin. Frightening by a dog also caused good dilatation of the pupil on the side on which the superior cervical ganglion had been removed, as well as of the normal pupil. In certain animals the dilatation produced by fright was sometimes even greater than in the normal eye, although in other observations on the same animals it might be less. In other animals the dilatation although quite marked was never so great as in the normal eye.

When the animals were etherized the pupil of the denervated eye in every case dilated more widely than that of the normal eye. The same was true when a moderate degree of asphyxia was produced. In some of the animals repeated observations were made on the effect of fright, asphyxia, and etherization at different periods after the operation up to 5 weeks, always with the same result. In one of the cats (No. 14) an experiment was eventually made to determine whether any detectable amount of epinephrin was being given off in the blood of the adrenal vein, with an entirely negative result.[5]

No difference could be seen in the pupil and pilomotor reactions between these animals and control cats in which one superior cervical ganglion had been excised without interference with the adrenals.

In cats our observations on the effect of morphine upon the epinephrin store of the adrenals agree with Elliott's. For example, in Cat 15 the connections of the left semilunar ganglion were cut and 5 days later 50 mg. of morphine were injected. After 8 hours the cat was killed. The left adrenal weighed 0.212 gm. and contained 0.24 mg. of epinephrin. The right adrenal weighed 0.209 gm. and contained 0.14 mg. of epinephrin.

In interpreting the depletion of the store seen after the long continued action of morphine, ether, urethane, and other anesthetics the possibility must not be lost sight of that the rate of formation or of accumulation of epinephrin in the adrenal may be diminished by the drugs. This would cause depletion of the store if the rate of liberation continued unchanged, just as surely as an increased rate of discharge would cause depletion if the rate of formation remained the same.

Is the Depletion of the Epinephrin Store under the Influence of β-Tetrahydronaphthylamine in Cats Due to Fright?—Elliott has shown that in the cat β-tetrahydronaphthylamine causes marked depletion of the epinephrin store of an adrenal whose nerve supply is intact as compared with its fellow whose nerve supply has been previously severed. We can confirm this statement. For example, in Cat 16, 3 days after section of the nerve supply on the left side 3 cc. of a 2 per cent solution of the drug were injected. After 8 hours the cat was killed. The left adrenal weighed 0.240 gm. and contained 0.22 mg. of epinephrin. The right adrenal weighed 0.200 gm. and contained only a trace of epinephrin.

Elliott associates the exhaustion of the epinephrin store in the cat with the emotional alarm indicated by the behavior of the animal, the wide dilatation of the pupil, etc. Having found reason to doubt the interpretation of the morphine effect on cats as due to emotional disturbance, the obvious suggestion occurred to us to try the effect of β-tetra upon the epinephrin store in the rabbit, an animal in which, according to Mutch and Pembrey,[6] the symptoms "give the impression

[6] Mutch, N., and Pembrey, M. S., *J. Physiol.*, 1911, xliii, 109.

that the drug produces a state of increased psychic activity accompanied by muscular action appropriate to the emotions."

It did not prove easy, however, to devise an operation which gave with constancy a good differential effect on the two adrenals. According to Kahn[7] and to Nishi,[8] the right adrenal in the rabbit seems to derive from the left splanchnic a portion of the nerve supply concerned in changes in the epinephrin store and in the liberation of epinephrin. Nishi was led to this conclusion by investigations on the glycosuria and hyperglycemia caused by diuretin, and Kahn by the results of his experiments on the relation of the adrenals to puncture glycosuria.

We tried a number of different operations to see whether such a differential effect on the epinephrin store as that described in the section on the postoperative deficit, or as that caused by urethane, ether, and morphine in cats and dogs, or by β-tetra in cats could be produced.

TABLE VI.

Experiments on Rabbits in Which the Right Major and Minor Splanchnics Were Divided.

No. of animal.	Weight of adrenal.		Epinephrin.		Time between operation and death.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
1	0.176	0.152	0.16	0.14	14 days.	Killed without drugs.
2	0.160	0.160	0.10	0.10	11 "	" " "
3	0.362	0.300	0.23	0.23	7 hrs.	" " "
4	0.290	0.240	0.13	0.12	14 days.	3 cc. 2 per cent β-tetra, 8 hrs. before being killed.
5	0.250	0.224	0.11	0.10	11 "	5 cc. 2 per cent β-tetra, 7 hrs. before being killed.
6	0.175	0.180	0.10	0.11	14 "	50 mg. morphine, 8 hrs. before being killed.

Table VI shows the results of experiments in which the right major and minor splanchnics were divided in the abdomen. As will be seen, the results were negative. Even in Rabbit 3, which was killed 7 hours after the operation, the content was precisely the same in the two adrenals. In other words, there was no postoperative deficit in the left adrenal after section of the right major and minor

[7] Kahn, R. H., *Arch. ges. Physiol.*, 1911, cxl, 209.

[8] Nishi, M., *Arch. exp. Path. u. Pharm.*, 1909, lxi, 401.

TABLE VII.

Experiments on Rabbits in Which the Left Major and Minor Splanchnics Were Divided.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
7	0.390*	0.250	0.13	0.14	3 days.	Major and minor splanchnics cut. 50 mg. morphine, 8 hrs. before being killed.
8	0.280	0.270	0.11	0.10	1 day.	Major and minor splanchnics cut, and 1 ganglion near lumbar vein cut out. Died spontaneously.
9	0.400	0.280	0.20	0.13	14 days.	Major and minor splanchnics and strand from lumbar chain cut. 70 mg. morphine in 2 doses, 9 hrs. before being killed.
10	0.276	0.218	0.17	0.18	5 "	Major and minor splanchnics cut. 5 cc. 2 per cent β-tetra, 6 hrs. before being killed.
11	0.309	0.270	0.18	0.16	3 "	Major and minor splanchnics cut. 5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
12	0.296	0.270	0.11	0.11	11 "	Major and minor splanchnics torn from origin through diaphragm and first 2 ganglia below diaphragm excised. 5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
13	0.250	0.216	0.20	0.14	12 "	Major and minor splanchnics, 2 ganglia, and strand from chain cut. 5 cc. β-tetra, 6 hrs. before being killed.
14	0.300	0.300	0.20	0.20	14 "	Major and minor splanchnics and 1 strand cut. 5 cc. β-tetra, 9 hrs. before being killed.
15	0.220	0.180	0.18	0.16	14 "	Major and minor splanchnics and 2 ganglia cut. 5 cc. β-tetra, 8 hrs. before being killed.
16	0.200	0.161	0.16	0.15	3 "	Left semilunar ganglion excised. 5 cc. β-tetra, 8 hrs. before being killed.
17	0.116	0.104	0.11	0.10	½ hr.†	Major and minor splanchnics cut. Killed as control.

* Edema of left adrenal.

† From beginning of anesthesia till end of operation, 25 minutes. Animal killed ½ hr. after operation. The time is too short for a decided postoperative effect.

splanchnics, the operation affording no protection to the epinephrin store of the right gland as compared with that of the left.

The Epinephrin Store in Postoperative Edema of the Adrenal.—Division of the left major and minor splanchnics was next performed on a series of rabbits. Sometimes, in addition, strands seen coming from the lumbar chain were divided and lumbar ganglia excised. The results are shown in Table VII. They are complicated to a considerable extent by the fact that edema, to which the rabbit's adrenals seem to be susceptible after operations in their vicinity, developed in some of the experiments. This edema is associated with great depletion of the epinephrin store of the affected gland. After a time the edema disappears and the epinephrin reaccumulates. It is obvious that if observations, on the effect of morphine or of β-tetra for example, are made on an animal in which edema of the left adrenal is still present, a genuine diminution in the epinephrin content of the right gland may be completely masked. In spite of this complication, however, Table VII indicates that section of the left major and minor splanchnics does, in some rabbits at least, produce a real differential effect, in contrast to the entirely negative results on section of these nerves on the right side shown in Table VI.

Thus, in Rabbit 9, which received morphine 14 days after the operation the left adrenal contained 0.20 mg. and the right only 0.13 mg. It is practically certain that the other morphine experiment in the table (Rabbit 7) would have shown a similar result but for the edema of the left gland, which was marked. Control observations prove that a gland with this degree of edema, 3 days after operation, never contains nearly so much epinephrin as its fellow. There is, therefore, every reason to believe that before the morphine was given the content of the right adrenal in this animal was considerably higher than that of the left. Since after the morphine period there is practical equality in the two glands, a considerable depletion of the store must be assumed to have taken place. In the dog and cat we have not seen the occurrence of edema of an adrenal in consequence of an operation in its neighborhood. Nor was there any edema in the right adrenal of the rabbit in the experiments in which the right major and minor splanchnic nerves were severed, possibly because the nerves on the right side were divided somewhat farther from the gland than those on the left side and there was accordingly less risk of interference with the lymphatics of the right gland. It is also possible that the mere section of the nerves is a factor in the development of edema, and if the right adrenal in the rabbit derives part of its innervation from the left splanchnics, division of the right splanchnics alone would not be so likely to affect it.

We next tried division of the fibers between the left semilunar ganglion and the adrenal, combined with free separation of the gland from the surrounding connective tissue by passing a blunt dissecting instrument around the greater part of its circumference, in the hope of destroying most of the innervation of the left adrenal while leaving intact such part, if any, of the innervation of the right adrenal as may come from the left splanchnic. As expected, edema of the gland developed, with the concomitant decrease in the epinephrin content already alluded to, only the merest trace being sometimes found. When a sufficient interval was allowed to elapse the edema cleared up and the store of epinephrin was replenished. The results are shown in Table VIII. The marked postoperative deficit seen in Rabbit 22, killed 5 hours after the operation, shows that the left gland was well protected relatively to the right by the operation. Edema of the left had not had time to develop in the few hours which had elapsed. In Rabbit 21, killed half an hour after the operation, no postoperative deficit was shown, the time being too short. This indicates that the marked depletion of the stock of epinephrin through the splanchnic nerves which follows surgical operations develops gradually. A number of the animals were killed as controls to determine the time necessary for recuperation of the epinephrin store.

In 7 days (Rabbit 18) the content of the left adrenal was not yet equal to that of the right. In 4 days (Rabbit 20) the depletion associated with the edema had reached its maximum, only a trace of epinephrin being present in the left adrenal. In 1 day (Rabbit 19), on the other hand, the left adrenal contained if anything, rather more than the right, the postoperative effect on the right (innervated) gland in all probability having not yet entirely disappeared, and the edema effect on the left not having attained its maximum, as is indicated by the relatively small excess of weight of the left gland. In Rabbit 28, which, like Rabbit 20, showed marked edema of the left gland after 4 days, no epinephrin reaction whatever was obtained from that gland.

Such observations as were made with β-tetra and with morphine and other drugs on animals prepared in this way did not yield decisive results for our immediate purpose, as, in the absence of a sufficient number of control animals kept for several weeks, it is not possible to be sure that equality in the epinephrin content of the two glands had been reached before the drugs were administered.

The results, however, seem worthy of being recorded because of the way in which they illustrate the depletion of the epinephrin store

TABLE VIII.

Experiments on Rabbits in Which the Nerves between the Left Semilunar Ganglion and the Gland Were Divided and the Gland Was Separated from the Surrounding Tissue.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
18	0.304	0.173	0.22	0.33	7 days.	Killed without drugs.
19	0.170	0.134	0.13	0.11	1 day.	" " "
20	0.440	0.210	Trace.	0.24	4 days.	" " "
21	0.252	0.210	0.24	0.27	½ hr.*	" " "
22	0.140	0.146	0.11	Trace.	5 hrs.	" " "
23	0.154	0.120	0.12	0.12	15 days.	3 gm. urethane 6½ hrs. before being killed.
24	0.175	0.156	0.11	0.11	17 "	Ether for 2½ hrs. Died of ether.
25	0.390	0.330	0.20	0.15	4 "	5 cc. 2 per cent β-tetra. Died in 6 hrs.
26	0.180	0.122	0.14	0.12	7 "	3 cc. 2 per cent β-tetra, 8 hrs. before being killed.
27	0.190	0.152	0.21	0.21	14 "	3 cc. 2 per cent β-tetra, 8 hrs. before being killed.
28	0.310	0.200	0.00	0.11	4 "	50 mg. morphine, 8½ hrs. before being killed.
29	0.326	0.228	0.14	0.24	7 "	50 mg. morphine, 6 hrs. before being killed.

* From beginning of anesthesia till end of operation was ½ hr. Animal killed ½ hr. after operation.

by conditions leading to edema of the glands after complete or at least extensive section of their secretory nerves and the rate of recuperation of the store of the denervated glands. It is probable that if the animals were kept longer the operation could be used to make tests for differential effects on the two adrenals.

Finally we tried division of the nerves between the semilunar ganglion and the left adrenal, with, in addition, section of any strands seen coming from the lumbar sympathetic towards the adrenal and excision of one or two of the sympathetic ganglia next below the diaphragm. Care was taken to avoid, as far as possible, interference with the gland. This operation gave the best results as regards constancy of effect, although even here the marked differences so easily obtained in dogs and cats were not seen.

TABLE IX.

Experiments on Rabbits, in All of Which the Nerves Were Divided between the Left Semilunar Ganglion and the Adrenal without Disturbance of the Gland. In Some of the Animals Portions of the Lumbar Sympathetic Chain Were Also Cut or Excised.

No. of animal.	Weight of adrenal.		Epinephrin.		Time after operation.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
30	0.324	0.276	0.18	0.14	14 days.	5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
31	0.300	0.260	0.24	0.20	10 "	5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
32	0.188	0.155	0.15	0.13	9 "	2 ganglia nearest diaphragm excised. 5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
33	0.140*	0.103	0.08	0.08	21 "	Strands crossing aorta cut. 5 cc. 2 per cent β-tetra, 8½ hrs. before being killed.
34	0.380	0.400	0.22	0.22	20 "	1 ganglion just below diaphragm excised. 5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
35	0.220	0.182	0.20	0.16	11 "	1 ganglion just below diaphragm excised. 5 cc. 2 per cent β-tetra, 8 hrs. before being killed.
36	0.356	0.314	0.20	0.16	8½ hrs.	Strands crossing aorta cut. Killed as control.

* In Rabbit 33 the left adrenal was found bound down by firm adhesions.

Table IX illustrates the findings. In Rabbit 36, killed to determine whether a postoperative deficit had been established in the right (innervated) gland, a positive result was obtained, although the difference was by no means as large as is often found in rabbits when one adrenal is excised and the other with its innervation intact left in for several hours longer (Table XI).

A number of experiments were made with β-tetra on animals prepared in this way. While in most cases some deficiency in the epinephrin content of the still innervated gland was found after the action of β-tetra, it was never nearly so great as in cats.

In Rabbit 33 the low content (0.08 mg.) in both adrenals is worth noting. The left gland was considerably larger than the right and was still, although 3 weeks

had elapsed since the operation, bound down by adhesions. It is probable, therefore, that the epinephrin store had not yet reached the initial amount. If so, since the right gland also contained only 0.08 mg. after β-tetra had acted for 8½ hours, the drug must have caused some depletion in the unprotected right adrenal. In cats after Elliott's operation on the connections of the semilunar ganglion, we have not seen any edema, and, as he states, equality of the epinephrin content of the two glands is soon established. In one cat (No. 17), however, which was killed as a control, 11 days after the operation on the nerves of the left adrenal, the gland was found firmly bound down by adhesions and the epinephrin content was decidedly less than that of its fellow. The left adrenal weighed 0.398 gm. and contained 0.22 mg. of epinephrin. The right adrenal weighed 0.371 gm. and contained 0.33 mg. of epinephrin.

This is a very exceptional result and was probably due to the fact that we happened to cut a vein in the vicinity, and in securing it some dissection was needed. Such an experiment would, of course, be rejected as a control.

As regards the question raised concerning the way in which β-tetra causes exhaustion of the epinephrin store in cats, it is evident that these observations do not lead us to the same definite conclusion as in the question concerning the mode of action of morphine. The results, indeed, might be interpreted as in favor of Elliott's view that the emotional disturbance in the cat is the efficient factor, since signs of such disturbance are less marked in the rabbit.

It must be kept in mind, however, that the rabbit has a more limited range in the expression of emotion than the cat, and β-tetra certainly produces excitation of sympathetic and other mechanisms which may be concerned in this expression. The pupil is widely dilated, the pulse and especially the respiration are markedly accelerated. There is cutaneous vasoconstriction, the ears being distinctly cool and the ear vessels narrowed. Usually the animals show restlessness and sometimes stamp with the hind feet. The rectal temperature in our observations rose 0.7–1°C.

The contrast between a rabbit under morphine and a rabbit which has received β-tetra is great. Yet it is easier to demonstrate diminution of the epinephrin store in the rabbit by morphine than by β-tetra. While our observations on morphine seem to indicate clearly that fright is not an essential factor in the morphine depletion, it would be unwarranted to claim that our observations on β-tetra show that fright is not the cause of the epinephrin depletion, so convincingly demonstrated by Elliott in the cat, under the influence of that drug.

It may be permissible to point out, however, that one drug (morphine) which causes different emotional phenomena in the cat and dog produces the same effect on the epinephrin store, while another drug (β-tetra) which causes in the cat and rabbit emotional effects of the same general quality and, perhaps, if the difference in the emotional development of the two animals is taken into account, not so very unequal in degree, affects the epinephrin store very differently.

Comparison of the Epinephrin Load of the Two Adrenals in Rabbits when Removed at Different Times.

Some experiments on rabbits were made in which the left adrenal was first excised, and the right subsequently, after varying periods of time. The results are shown in Table X. It will be seen that in the five animals killed without drugs (Nos. 37, 38, 39, 40, and 41) the right adrenal contains more epinephrin than the left contained at the time of excision. Where the difference is small it could be accounted for

TABLE X.

Experiments on Rabbits to Determine the Load of the Right Adrenal at Varying Intervals after Removal of the Left.

No. of animal.	Weight of adrenal.		Epinephrin.		Interval between excision of 2 adrenals.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*	*days*	
37	0.454	0.470	0.22	0.28	14	Killed without drugs.
38	0.162	0.190	0.10	0.13	14	" " "
39	0.312	0.324	0.14	0.22	17	" " "
40	0.420	0.346	0.16	0.33	26	" " "
41	0.270	0.176	0.13	0.17	18	Abscess at site of operation, but superficial. Killed without drugs.
42	0.158	0.164	0.16	0.16	16	50 mg. morphine, 7 hrs. before removal of right adrenal.
43	0.450	0.640	0.22	0.47	16	5 cc. 2 per cent β-tetra, 9 hrs. before removal of right adrenal.
44	0.288	0.350	0.18	0.33	16	5 cc. 2 per cent β-tetra, 9 hrs. before removal of right adrenal.

by the diminution of the epinephrin load in both adrenals even during the short operation necessary for removal of the left adrenal. But such a difference as that seen in Rabbit 40, in which the interval between the removal of the two glands was greatest is too large to be explained in this way. In Rabbit 44 this factor was taken into account by removing the second adrenal by an operation which in duration and technique as nearly as possible duplicated the first operation. It must, therefore, be concluded that the load of the remaining adrenal in most of the animals is increased above its amount at the time of excision of the first gland. More observations, especially with longer intervals, would be necessary to make sure that the remaining adrenal can accumulate more epinephrin than is ever seen in one adrenal when both are present. A glance at the other tables which give results on rabbits will show that the number of high loads of the right adrenal in Table X is unusually great for the number of animals, and this is suggestive.

The experiments were originally undertaken with the idea that if the right adrenal regained only its initial content after the operation the animals could be employed for testing the action of substances like morphine or β-tetra on the epinephrin store. The fact that the load increased so much rendered this method too uncertain. The difference in the result in Rabbit 42 which received morphine, and Rabbits 43 and 44 which received β-tetra, is nevertheless striking. The preponderance of the content of the right adrenal in the last two experiments as compared with the left indicates strongly that the influence of β-tetra in discharging the right adrenal was at any rate not great. On the other hand, unless Rabbit 42 was the single exception among the eight animals in the table, some discharge of the right adrenal must have been occasioned by the morphine, since the content after morphine was just the same as that of the left adrenal.

Postoperative Depletion of the Epinephrin Store.

The fact has already been alluded to that during and for some time after operations a more or less progressive decline takes place in the epinephrin store of an adrenal whose nerve supply is intact as compared with its denervated fellow. The phenomenon was well illustrated in experiments in which one adrenal was removed, and then after varying intervals of time the other. The exact opposite of the results shown in Table X is then seen; that is, a marked deficiency in the second adrenal as compared with the first. For example, in

Rabbit 45 (Table XI) the left adrenal was excised and contained 0.37 mg. of epinephrin. The wound was sewed up, the whole operation lasting 20 minutes, and after 8½ hours the right adrenal was removed. It contained only 0.13 mg. of epinephrin.

TABLE XI.

Experiments Showing Postoperative Depletion of Epinephrin Store of One Adrenal as Compared with the Other Removed at Operation.

No. of animal.	Weight of adrenal.		Epinephrin.		Interval between excision of 2 adrenals.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*		
Rabbit 45.....	0.394	0.384	0.37	0.13	8½ hrs.	
Cat 18.....	0.220	0.218	0.18	0.14	5 "	
" 19.....	0.205	0.238	0.22	0.25	2 days.	
Rabbit 46.....	0.318	0.314	0.20	0.11	6½ hrs.	5 cc. 2 per cent β-tetra, 6½ hrs. before being killed.
" 47.....	0.092	0.084	0.14	0.08	7 "	50 mg. morphine, 7½ hrs. before being killed.
" 48.....	0.267	0.238	0.11	0.09	6½ "	50 mg. morphine, 6½ hrs. before being killed.
" 49.....	0.380	0.408	0.28	0.12+	8 "	50 mg. morphine, 8 hrs. before being killed.
Cat 20.....	0.240	0.300	0.33	0.20	6 "	50 mg. morphine, 6 hrs. before being killed.
" 21.....	0.225	0.258	0.22	0.14	7 "	50 mg. morphine, 7 hrs. before being killed.
" 22.....	0.230	0.221	0.26	0.26	8 "	Right adrenal excised and nerves of left adrenal cut. 50 mg. morphine 8 hrs. before being killed.
Dog. 14.....	0.354	0.453	0.67	0.42	8 "	175 mg. morphine 8 hrs. before being killed.
" 15.....	0.352	0.360	0.38	0.30	7 "	200 mg. morphine 7 hrs. before being killed.

This great depletion was assuredly not present at the end of the operation, as shown in other experiments. Etherization for 20 or 30 minutes does not produce a great impression on the store of an adrenal removed at the end of that time. Unless, then, the depletion

is an after-effect of the short etherization, which does not seem likely, it must be due to some other factor, probably the sensory stimulation acting during the postoperative period.

Similar results in animals with one gland protected by section of its nerves have already been given in other tables; for example, Rabbit 22 (Table VIII). 5 hours after section of the nerves of the left adrenal, the animal was killed. The right adrenal contained only a trace of epinephrin, the left 0.11 mg. In the same table and in Table VII examples are given where in half an hour after the operation practically no postoperative difference was present.

The recuperation of the store is usually evident in 1 day, although it may not then be complete. For instance, in Cat 19 (Table XI), 2 days after removal of the left adrenal the right contained 0.25 mg. as compared with 0.22 mg. in the left. Probably the difference represents about the amount by which the store of the left adrenal was diminished during the short operation for its removal. Many of the animals received morphine immediately after excision of the first adrenal (always the left except in Cat 22), and one (Rabbit 46) received β-tetra. The effect, if any, of these drugs is complicated by the postoperative depletion.

Cat 22 shows how completely section of the nerve supply of an adrenal protects the store even against the combined effect of the operation and morphine. All preparations having been made to insure almost simultaneous removal of one adrenal and denervation of its fellow, the right adrenal was excised in 5 minutes, and the nerves of the left adrenal were cut within the next 2 minutes. Even after 8 hours, the epinephrin content of the left adrenal was precisely the same as that of the right; namely, 0.26 mg.

In Table XII are given the results of assays of the adrenals in a number of experiments on the liberation of epinephrin into the blood.[5] All except the first show a relatively low content, since the nerves were cut in the course of the experiment, usually some hours after the beginning. Where the innervation of one adrenal is divided after several hours of experimentation, so great a depletion of the epinephrin store of both glands has already taken place that subsequent division of the nerves of the other, an hour or two later, does not generally establish such a striking difference between the two glands as when the nerves on one side are severed at the beginning.

In Table XIII are shown the epinephrin assays of the adrenals obtained in other experiments on liberation of epinephrin. In these the innervation of the glands was not interfered with. Equality of content in the two adrenals of the same animal is well shown. While there are, of course, variations in the different animals, the average content is lower than that of the cats in Tables I and II.

TABLE XII.

Depletion of the Epinephrin Store of the Innervated Adrenal during Physiological Experiments.

No. of animal.	Weight of adrenal.		Epinephrin.		Duration of experiment.	Remarks.
	Left.	Right.	Left.	Right.		
	gm.	*gm.*	*mg.*	*mg.*	*hrs.*	
Cat 23.....	0.260	0.286	0.18	0.25	5	Connections of right semilunar ganglion cut 11 days before experiment.
" 24.....	0.230	0.230	0.17	0.28	2½	Connections of right semilunar ganglion cut 15 days before experiment. Used for liberation experiment and died during the experiment. In poor condition; abortion the day before.
" 25.....	0.204	0.211	0.10	0.17	5	Right major and minor splanchnics cut 3½ hrs. after beginning of experiment.
" 26	0.197	0.201	0.14	0.16	5½	Right sympathetic (in thorax) cut 2½ hrs. after beginning of experiment. Left sympathetic cut 2¼ hrs. later.
" 27.....	0.140	0.140	0.11	0.11	3¾	Right sympathetic (in thorax) cut 3 hrs. after beginning; left sympathetic cut ¾ hr. later.
" 28.....	0.204	0.216	0.17	0.18	4	Both sympathetics cut in thorax, 2½ hrs. after beginning.
" 29.....	0.120	0.122	0.18	0.18	2½	Both sympathetics cut 1 hr. after beginning.
" 30.....	0.221	0.228	0.13	0.14	6	Both sympathetics cut 5 hrs. after beginning. 1 hr. later, adrenals massaged.
" 31.....	0.242	0.234	0.18	0.16	4½	Both sympathetics cut 3 hrs. after beginning.
" 32.....	0.185	0.200	0.11	0.09	5½	Both major splanchnics cut 4½ hrs. after beginning.
Dog 16.....	0.524	0.560	0.41	0.37	6¼	Right sympathetic (in thorax) cut 4½ hrs. after beginning, left sympathetic 1¼ hrs. later.

TABLE XIII.

Epinephrin Content in Cats Used for Experiments under Urethane and Ether without Section of Nerves to Adrenals.

No. of animal.	Weight of adrenal.		Epinephrin.		Duration of experiment.
	Left.	Right.	Left.	Right.	
	gm.	*gm.*	*mg.*	*mg.*	*hrs.*
33	0.178	0.164	0.14	0.14	5¼
34	0.294	0.290	0.17	0.16	4
35	0.226	0.224	0.20	0.19	3¾
36	0.256	0.238	0.20	0.19	4¾
37	0.330	0.364	0.18	0.20	5¾

Depletion of Epinephrin Store of the Innervated as Compared with That of the Denervated Adrenal in Animals, Dead of Infections, Etc.

The opportunity of making these observations was afforded by the death, from various causes, of a certain number of animals whose adrenal nerve supply had been cut on one side. The results are given in Table XIV. Complete or almost complete depletion of the epinephrin store of the still innervated gland was far more frequently observed in this group than in any of the experimental groups.

Infections of various kinds (pyogenic in Cats 38 and 41, pneumonia in Dogs 17 and 18) caused particularly marked depletion. It has been stated by a number of observers that the adrenals in animals killed by various infections are poor in epinephrin. Elliott[2] found a small epinephrin load in persons dead of various septic fevers. The results cited in Table XIV show clearly that this depletion is brought about, in great part at any rate, through the nerves supplying the adrenals. It is not possible to say from these results whether, in addition, there is any direct action of the poisons on the glands.

In a cat (No. 43) dead of distemper the epinephrin content was quite low (0.09 mg.), but in another cat (No. 42), dead of the same disease, the load was fairly high (0.28 mg.), although perhaps not as much as would have been expected in a healthy animal of the same size. The cat was exceptionally large, as were also the adrenals.

TABLE XIV.

Depletion of the Epinephrin Store of the Innervated Adrenal in Animals Dead from Infections, Etc.

No. of animal.	Weight of adrenal.		Epinephrin.		Remarks.
	Left.	Right.	Left.	Right.	
	gm.	*gm.*	*mg.*	*mg.*	
Cat 38.....	0.184	0.191	0.16	Trace.	Connections of left semilunar ganglion cut 8 days before death from infection (peritonitis).
" 39.....	0.312	0.304	0.09	"	Connections of left semilunar ganglion cut 5 days before death from unknown cause. Cat emaciated and in poor condition before operation.
" 40.....	0.170	0.198	Trace.	0.13	Connections of right semilunar ganglion cut 2 days before death from unknown cause. Cat in same condition as No. 39.
" 41.....	0.308	0.300	0.08	0.14	Connections of right semilunar cut 25 days before death. General infection; abscess in wound in neck and in flank; eyes infected.
" 42.....	0.396	0.402	0.28	0.28	Died of distemper 14 days after excision of left superior cervical ganglion.
" 43.....	0.278	0.272	0.09	0.09	Died of distemper 6 days after excision of left superior cervical ganglion.
Dog 17.....	0.530	0.570	0.50	0.31	Left major splanchnic cut 13 days before death from pneumonia.
" 18.....	0.360	0.350	0.30	0.08	Left major and minor splanchnics, 2 lumbar ganglia, and connections of celiac ganglion cut 14 days before death from pneumonia.
Rabbit 50.....	0.200	0.210	0.09	Trace.	Left adrenal excised 3 days before death from unknown cause.

The Effect of Electrical Stimulation of the Splanchnic Nerves on the Epinephrin Store.

Elliott has stated that stimulation of the splanchnics by induction shocks for periods varying from 3 to 7 hours produces only a slight effect on the epinephrin content. Stimulation was applied for a few minutes at a time followed by a few minutes of rest. "When the nerve was faradized continuously for a period of 2 hours the loss was almost inappreciable."

Our observations confirm those of Elliott, in as far as they show that it is much more difficult to demonstrate depletion by splanchnic

stimulation than under the influence of ether, operations, etc., despite the fact that more epinephrin is liberated in a given time during appropriate stimulation of the nerves than is liberated under anesthetics without stimulation. The difficulty of showing depletion with splanchnic stimulation is, therefore, not due to the small amount liberated. We cannot see any other explanation than that splanchnic stimulation also increases the rate of accumulation of the epinephrin in the gland.

Continuous stimulation of the splanchnic is not suitable for producing depletion, nor is stimulation for several minutes at a time with intervals of rest. For we have seen in experiments on liberation of epinephrin into the blood that the amount excreted rapidly declines when stimulation is kept up.

However, by stimulating only for a few seconds at a time, with intervals of 1 or 2 minutes' rest, and continuing the experiment over many hours we have been able to demonstrate distinct depletion of the gland whose nerve was excited as compared with the other. The reactions of the denervated eye were used to control the efficiency of the stimulation. A strength and duration of stimulation was chosen which gave good but not maximal dilatation of the pupil, and the rest intervals were increased or diminished from time to time, so as to keep each stimulation effective.

In Table XV is given a condensed protocol of an experiment on a cat in which over 300 successful stimulations of the right sympathetic trunk (with the splanchnic) in the thorax were made. The amount of epinephrin liberated by each stimulation was estimated by determining, at different times throughout the experiment, the amount of adrenalin which had to be injected to give approximately a pupil dilatation of the same magnitude, and beginning at the same time interval, as the dilatation caused by the liberated epinephrin. Both sympathetics were divided simultaneously in the thorax, so that the glands were under equal conditions, except for stimulation of the nerves of the right. At the end of the experiment the left adrenal weighed 0.159 gm. and contained 0.20 mg. of epinephrin, and the right adrenal weighed 0.181 gm. and contained 0.08 mg. of epinephrin.

The assays by adrenalin solution of the amount of epinephrin liberated from the right adrenal into the blood yielded the following results.

Epinephrin liberated by 316 stimulations, 0.31 mg.

Time.	No. of stimulations.	Adrenalin corresponding to each stimulation.	Total epinephrin.
		mg.	*mg.*
3.10–5.01 p.m.	47	0.002	0.09
5.04–7.28 p.m.	95	0.0013	0.12
7.32 p.m.–12.15 a.m.	174	0.0006	0.10

With the short time of each stimulation and the relatively long intervals of rest, it is to be supposed that the power of the adrenal to respond to stimulation of its nerves was conserved as much as possible. Nevertheless, the diminution in the response, which was not apparent for a long time during the earlier part of the experiment became more marked as time went on. This is strikingly illustrated in the table showing the amount of liberated epinephrin. The 174 stimulations in the last period yielded only 0.10 mg. of epinephrin, while forty-seven stimulations of the same duration (5 seconds) yielded in the first period of the experiment 0.09 mg. Add to this the fact that the strength of stimulation had to be considerably increased in the latter part of the experiment, as shown by the diminished distance between the coils given in the protocol.

The load of the right adrenal at the time the nerves were cut may be taken at 0.20 mg., the same as that of the left. This represents a good load for these small adrenals (the cat weighed only a little over 2 kilos), especially as some loss must have occurred in the hour after the administration of urethane before the nerves were divided. The right adrenal gave off to the blood 0.31 mg. of epinephrin, of which 0.12 mg. could have come from the store. This is on the assumption that no epinephrin whatever is liberated in the absence of innervation.[5] An amount approximately equal to the initial load must have been formed in the time the experiment lasted.

In other experiments lasting for a shorter time we have not been able to obtain evidence of depletion of the store by splanchnic stimulation. In one with over fifty stimulations, each of which caused the liberation of enough epinephrin to elicit good eye reactions, no deficiency in the store of the stimulated gland was found, although in the 4 hours of the experiment an amount of epinephrin not less than the maximal load of a single gland in the cat had been given off by it to the blood.

It is possible that in prolonged experiments the capacity of the adrenal to form epinephrin at an increased rate in response to splanchnic stimulation is lessened, and that this is the reason why depletion of the store can then be demonstrated.

TABLE XV.

Condensed Protocol. Cat 44, Weight 2.25 Kilos. Superior Cervical Ganglion Excised 17 Days before. Animal Pregnant. Cannulas in Right Femoral Vein and Trachea. Unless Otherwise Mentioned, Time of Stimulation Was Always 5 Seconds. The Left Sympathetic Was Stimulated Only Once.

Time.		Interval between beginning of stimulation and of pupil dilatation.
p. m.		*sec.*
1.40	Urethane, 3 gm. by stomach tube	
2.20	A little ether. No more ether during experiment	
2.45	Both sympathetics (with splanchnic) ligated and cut in thorax	
3.10	Stimulated left sympathetic (11 cm. between coils)	9.6
3.30–4.08	Stimulated right sympathetic (3–5 sec.) (9–10 cm.) 10 times	6.6–7.8*
4.08	Adrenalin injections. Same pupil reactions given by 0.3 cc. (1 : 140,000)†	
4.09–4.27	Stimulated right sympathetic (9 cm.) 15 times	6.0–7.2
4.27	Adrenalin injections. Reaction, 0.3 cc	
4.28–4.36	Stimulated right sympathetic (9 cm.) 6 times	7.4–8.8
4.36	Adrenalin injections. Reaction, 0.3 cc	
4.40–4.46	Stimulated right sympathetic (9 cm.) 5 times	7.6–8.2
4.47	Adrenalin injections. Reaction, 0.3 cc	
4.49–5.01	Stimulated right sympathetic (9 cm.) 11 times	6.8–7.8
5.02	Adrenalin injections. Reaction, 0.3 cc	
5.04–5.21	Stimulated right sympathetic (9 cm.) 14 times	7.2–8.0
5.22	Adrenalin injections. Reaction, 0.25 cc	

*The time interval of the pupil reaction was constant throughout the experiment. Only the minimum and maximum times are given in this column for the various series of stimulations. The times for the pupil reactions yielded by these 10 stimulations may be given as an example (7.4, 7.0, 7.4, 7.8, 7.8, 7.2, 7.2, 7.6, 6.6, 7.2). The number in parenthesis after stimulation gives the distance between the coils in cm.

†Only the quantities of adrenalin found in the trial injections to correspond with the effects of the splanchnic stimulations are reproduced from the protocol. The adrenalin solution was itself assayed. The corrected concentration is what is given in the protocol. A freshly made solution in 0.9 per cent sodium chloride was used for each assay. The adrenalin solution injected was always a 1:140,000. The concentration of the solution is therefore henceforth omitted in the protocol.

TABLE XV—*Concluded.*

Time.		Interval between beginning of stimulation and of pupil dilatation.
p. m.		*sec.*
5.25–5.36	Stimulated right sympathetic (9 cm.) 8 times	7.2–8.2
5.38	Adrenalin injections. Reaction, 0.2 cc.	
5.40–5.59	Stimulated right sympathetic (9 cm.) 15 times	6.6–8.6
6.00	Adrenalin injections. Reaction, 0.2 cc.	
6.02–6.18	Stimulated right sympathetic (9 cm.) 13 times	6.8–7.4
6.20	Adrenalin injections. Reaction, 0.2 cc.	
6.22–6.36	Stimulated right sympathetic (9 cm.) 12 times	6.4–7.2
6.40	Adrenalin injections. Reaction, 0.2 cc.	
6.42–6.53	Stimulated right sympathetic (9 cm.) 7 times	7.0–7.4
6.58	Adrenalin injections. Reaction, 0.2 cc.	
7.00–7.28	Stimulated right sympathetic (9 cm.) 26 times	6.6–7.2
7.31	Adrenalin injections. Reaction, 0.2 cc.	
7.32–7.50	Stimulated right sympathetic (9 cm.) 18 times	6.2–7.2
7.54	Adrenalin injections. Reaction, 0.1 cc.	
7.55–8.10	Stimulated right sympathetic (8 cm.) 13 times	6.2–8.8
8.12	Adrenalin injections. Reaction, 0.1 cc.	
8.15–8.38	Stimulated right sympathetic (8 cm.) 20 times	6.4–7.8
8.40	Adrenalin injections. Reaction, 0.1 cc.	
8.42–9.20	Stimulated right sympathetic (7.5 cm.) 26 times	6.4–7.8
9.22	Adrenalin injections. Reaction, 0.1 cc.	
9.26–9.48	Stimulated right sympathetic (7.5 cm.) 14 times	6.8–8.0
9.50	Adrenalin injections. Reaction, 0.1 cc.	
9.53–10.25	Stimulated right sympathetic (7.5 cm.) 24 times	6.8–7.8
10.27	Adrenalin injections. Reaction, 0.1 cc.	
10.30–10.50	Stimulated right sympathetic (7.5 cm.) 16 times	6.4–7.8
10.53	Adrenalin injections. Reaction, 0.1 cc.	
10.55–11.30	Stimulated right sympathetic (7.5 cm.) 23 times	6.8–8.8
11.34	Adrenalin injections. Reaction, 0.1 cc.	
11.37 p.m.–12.15 a.m.	Stimulated right sympathetic (7.5 cm.) 20 times	6.8–9.0
12.15 a. m.	Adrenalin injections. Reaction, 0.1 cc.	

Circulation good up to the end of the experiment; the heart was beating well, although the pulse was gradually becoming more rapid toward the last hour of the experiment.

The left adrenal weighed 0.159 gm. and contained 0.20 mg. of epinephrin.

The right adrenal weighed 0.181 gm. and contained 0.08 mg. of epinephrin.

SUMMARY.

1. No evidence has been obtained that in cats and dogs with the nerves of one adrenal cut, emotional disturbances cause depletion of the epinephrin store of the normally innervated adrenal as compared with its fellow.

2. The depletion of the epinephrin store in cats under morphine is not dependent upon so called morphine fright, since a similar depletion is found in dogs in which, as is known, morphine produces symptoms the reverse of those of fright.

3. The signs of morphine fright can all be elicited by administering morphine to a cat in which one adrenal has been removed and the nerve supply of the other cut, and in which accordingly no detectable liberation of epinephrin takes place.

4. The reactions of the denervated iris elicited by emotional disturbance, asphyxia, or etherization in a cat, one of whose adrenals has been removed and the nerves of the other cut, do not differ from these reactions in cats whose adrenals have not been interfered with.

5. The influence of postoperative edema of the adrenal in diminishing the epinephrin load, and the recuperation of the load after a time, have been studied in rabbits.

6. The diminution in the epinephrin store of the adrenals which follows operations on animals (postoperative depletion) has been studied. It is only in part associated with the anesthesia, since it may be as marked 6 or 8 hours after an operation lasting less than 1 hour as after 6 or 8 hours' anesthesia without operation.

7. One adrenal was removed in rabbits and the epinephrin content of the remaining gland assayed at varying periods of time after removal of the first, the periods being longer than the time necessary for recovery from the postoperative depletion. In general, the second adrenal contained more epinephrin than the first, sometimes double the amount.

8. Marked depletion of the epinephrin store of innervated adrenals as compared with the corresponding denervated glands was seen in animals dead of infections of various kinds.

9. As shown by Elliott, diminution of the stock of epinephrin in the adrenal through electrical stimulation of the splanchnics is not easy to demonstrate, despite the fact that the liberation of epinephrin into the blood is notably increased by the stimulation. With short periods of stimulation, however, repeated over a long time at intervals just long enough to prevent fatigue, it has proved possible to demonstrate a distinct depletion.

A STUDY OF EXPERIMENTAL NON-HEMOLYTIC STREPTOCOCCUS LESIONS IN VITALLY STAINED RABBITS.

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PLATES 49 TO 51.

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Within the last 15 years much has been written about the streptococcus in rheumatic fever, and emphasis has been laid on the bacteriological rather than on the pathological aspects of the disease. The reasons for this were twofold: (1) the low mortality in rheumatic fever made the material for pathological study scanty; (2) the frequently reported occurrence of streptococci in the various lesions of rheumatic fever cases stimulated so much interest in the bacteriology of the disease that other phases of the problem have been neglected.

HISTORICAL.

The most important recent contribution to the pathology of rheumatic fever is that of Aschoff (1), who in 1904 described certain small collections of cells in the myocardium which he considered characteristic of rheumatic fever. Aschoff spoke of them as "submiliary nodules." They are usually found in the neighborhood of the smaller arterioles and consist of a collection of unusually large cells with one or more large irregularly shaped nuclei. These giant cells, according to Aschoff, are often arranged like a rosette about a center of necrotic confluent cellular protoplasm. Just outside the giant cells, which resemble somewhat those seen in Hodgkin's disease, there is a peripheral zone of leukocytes, in which lymphocytes predominate. The peripheral cells form streaks or out-shoots into the connective tissue septa. In these streaks are also found the adventitia or wandering cells which appear in all inflammatory processes. They arise from the vessels and it is from them, Aschoff thinks, that the giant cells develop.

In the following year Geipel (2) studied five cases of rheumatic myocarditis. His description of the submiliary nodule agrees with Aschoff's, but he disagrees with Aschoff as to the origin of the giant cells, for he believes that they arise from connective tissue. According to Geipel, the connective tissue cells swell

and in some instances fuse together to form the multinucleated giant cells. Both Aschoff and Geipel describe the gradual conversion of the submiliary nodule into a patch of fibrous tissue. Aschoff claims that these foci are specific for rheumatic fever, but Geipel will not admit this.

Fraenkel (3) confirmed Aschoff's observations as to the specificity of the nodules, but does not commit himself as to the origin of the giant cells. Coombs (4) has devoted considerable study to Aschoff's bodies, and, like Geipel, considers them "the product of a connective-tissue proliferation in response to an irritant."

Bracht and Wächter (5) found the Aschoff bodies in three out of four cases of rheumatic myocarditis. They also conclude that the giant cells are derived from the fixed connective tissue cells, basing their opinion on the fact that these cells stain with the Unna-Pappenheim stain like swollen fibroblasts. These investigators were the first to produce experimental focal lesions in the myocardium of rabbits by injecting them with streptococcus. Rabbits injected with *Streptococcus viridans* showed considerable necrosis of the cardiac muscle fibers. The older foci showed a considerable number of lymphocytes and fibroblasts and a few giant cells. In still older lesions, fibrous scars were forming. In animals, on the other hand, that were injected with *Streptococcus hemolyticus*, the heart muscle showed small abscesses and dense infiltration of polynuclear leukocytes.

Takayasa (6) describes the heart changes in one case of rheumatism in which he found Aschoff bodies, but he offers no theory as to the origin of the giant cells.

Thalhimer and Rothschild (7) have recently studied the cardiac changes in rheumatic fever and chorea and conclude that the presence of Aschoff nodules signifies a previous rheumatic infection.

Jackson (8) injected hemolytic streptococci and also a *Streptococcus viridans* into rabbits. With the latter she produced foci consisting of degenerated muscle fibers around which are large mononuclear cells, large multinucleated giant cells, and proliferating connective tissue. In other lesions "the cells occupying the center of the lesion are very large with round or oval lightly stained nuclei and a large amount of granular cytoplasm in which bacteria are often seen." About these cells are many smaller, more deeply stained cells which are irregularly shaped and definitely outlined, with round deeply stained eccentric nuclei (plasma cells). In the relative absence of necrosis and of polynuclear leukocytes and in the abundance of large endothelial cells, these lesions are peculiar, showing the same characteristics emphasized by Coombs and others as characteristic of the focal human lesions in rheumatism.

De Vecchi (9) in 1912 injected the blood from cases of rheumatic fever into rabbits and was able thereby to produce lesions in the heart muscle. The lesions consisted of diffuse infiltrations of cells usually about a small vessel. The muscle fibers are often devoid of contractile tissue and appear homogeneous or in amorphous blocks. The cellular foci are composed of (1) leukocytes, mostly lymphocytes, (2) cells with large vesicular nuclei containing a network of chromatin and

scanty protoplasms. De Vecchi remarks that these experimental lesions resemble the rheumatic nodules, but his description and drawings do not support this claim. They might more readily be explained as a result of the injection of a foreign protein, as shown by Longcope (10).

Coombs, Miller, and Kettle (11) inoculated rabbits with streptococcus and produced cardiac lesions which differed in no essential manner from the lesions of human rheumatism.

Schloss and Foster's (12) experiments on monkeys were performed with *Streptococcus hemolyticus;* hence they are hardly comparable with the experiments which have been described above. They found the myocardial vessels thickened and surrounded in places by groups of cells which took a deep red color with pyronin methyl green. These cells looked like plasma cells, but were a little larger. Their nuclei were eccentric and stained light blue or green. Thalhimer and Rothschild (7) injected rabbits with *Streptococcus mitis* (*viridans*) and produced focalized myocardial lesions which they describe as follows:

"The lesions are entirely different from the submiliary myocardial rheumatic nodules described by Aschoff. . . . . The lesions in the myocardium of the rabbit are a combination of degenerative and productive processes. Degeneration of the muscle fibres occurs either accompanied by or soon followed by a proliferation of the interstitial tissue which is disproportionate to the extent of the myocardial change."

The following facts are evident from this review of the literature. (1) Opinion is almost unanimous in considering Aschoff bodies as specific for rheumatic fever. (2) There is considerable diversity of opinion as to the origin and character of the large cells which for the most part constitute the lesion. By Coombs they are considered endothelial; by Geipel, Bracht and Wächter, and others, fibroblasts; while Aschoff himself looks upon them as transformed macrophages or wandering cells. (3) Efforts to reproduce Aschoff bodies experimentally have met with varying results in the hands of different investigators. Coombs and de Vecchi and Jackson all claim to have produced lesions which they consider essentially identical with Aschoff bodies. Bracht and Wächter, and Thalhimer and Rothschild produced lesions which are not identical with Aschoff bodies and are easily distinguished from them.

It is obvious that once granted that Aschoff bodies are a specific manifestation of rheumatic fever, the experimental production of them by injection of streptococci would be strong evidence in favor of the streptococcal origin of rheumatism. The present study was undertaken to determine, if possible, the origin of the peculiar cells

in the myocardial lesion; as the work progressed it seemed desirable to include also a study of the other structures involved, especially the joints.

In view of the disagreement as to the origin of the cells which constitute the Aschoff body in the human heart and the lack of harmony in the results from experimental study of the lesion, it was thought that the employment of a vital stain might prove of value.

Evans, Bowman, and Winternitz (13) have recently used this method with considerable success for the histological study of the miliary tubercle, and by vital stains demonstrated that in the liver both the epithelioid and giant cells of the tubercle develop from the endothelial lining of the capillaries.

In using the vital stain I have followed the method of Goldmann (14) who first reported his experiments with trypan blue and isamin blue in 1912. Goldmann employed the dyes in 1 per cent aqueous solutions and administered them either subcutaneously, intraperitoneally, or intravenously. In any case, the animals rapidly took on a deep blue color and at autopsy the various organs and tissues showed more or less pigmentation with the dye. Microscopically it was at once obvious that certain cells had taken up the blue granules, while others had not. For instance, Goldmann noted that the white corpuscles of the blood and the plasma cells never took the blue; on the other hand, the so called wandering cells, which are present throughout all the tissues, invariably showed phagocytosis of the granules. These wandering cells have received a variety of names, being variously known as macrophages, polyblasts, adventitia cells, clasmatocytes, or endothelioid cells, and Goldmann lengthened the list by calling them pyrrhol cells. He found them in the subcutaneous tissue and in all the internal organs and very abundant in the pregnant uterus and in healing wounds. The characteristic feature of these cells is their active phagocytosis of bacteria and foreign matter of any kind. Goldmann thinks that they eventually stretch to form the spindle cells of young connective tissue, thereby losing their affinity for vital and fat stains as well as their phagocytic power.

Evans (15) has recently described these vitally staining cells in detail. He speaks of them as macrophages and classifies them as follows: (1) endothelial macrophages which line the capillaries in the liver, spleen, bone marrow, and hemal glands, and the lymphatic sinuses of the lymph glands; (2) macrophages which are more or less fixed, such as the cells of the reticulum of lymph glands, spleen, and bone marrow; also resting wandering cells in the connective tissues, especially numerous in the omentum; (3) the free macrophages which comprise the large mononuclear cells of the serous cavities and those present in the lymphatic sinuses of lymph glands and sometimes seen in the hepatic and splenic capillaries. Evans has demonstrated that the intramuscular macrophages may develop from endothelium, but he is not willing to go so far as Mallory, who considers all macrophages of endothelial origin.

It will be seen from this review that the vital stain is of considerable value in the study of cellular foci as helping to distinguish the various types, leukocyte, endothelium, fibroblast, and macrophage from one another.

Methods.

In the present study, the technique employed was as follows: Freshly distilled water was heated to 56°C. and enough trypan blue added to make a 1 per cent solution. While still warm the dye was injected into the rabbits usually by the intravenous route, though occasionally subcutaneous or intraperitoneal injections were substituted. The dye has a tendency to produce thrombosis of the ear veins. The rabbits were usually injected first with streptococci, but in some cases the trypan blue was administered previously. Histologically it seemed to make no difference which preceded the other. The usual dose of trypan blue was 10 cc. of the 1 per cent solution every day for 1 to 2 weeks. The number of trypan blue injections varied from one to twenty-three, the average number being twelve. By this method it was found that the tissues of the rabbits acquired a deep blue color and at the same time the animals suffered little physical deterioration. Most of the rabbits were killed by intracardiac injection of formalin. The rabbit was anesthetized, and the jugular and femoral veins were exposed. The sternum was then rapidly removed and a cannula inserted into the left ventricle. A 4 per cent solution of formalin was then allowed to run in through the cannula and at the same time the jugular and femoral veins were severed. The animal was thus rapidly exsanguinated, and in a few minutes formalin could be detected in the venous outpourings.

The perfusion with formalin was continued for 10 to 15 minutes, usually until several hundred cc. of the solution had been injected. A complete autopsy was then performed and thin pieces of tissue were preserved in 4 per cent formalin for sections. Sections were also taken from the infected joints and decalcified in Müller's fluid. Films and sometimes cultures were made from the pus in the infected joints. The intracardiac injection of formalin did not interfere with the recovery of streptococci in culture from the joints.

Thirty-eight rabbits in all were used in the experiments (Table I). In addition, three controls were injected with trypan blue but received

TABLE I.

No. of rabbit.	Weight.	No. of trypan blue injections.	No. of streptococcus injections.	Duration of life after 1st injection of streptococci.	Arthritis.	Lesions in heart.	Lesions in liver.	Lesions in kidneys.	Remarks.
	gm.			*days*					
1	1,820	2	1 (59–F)	1	0	Focal necrosis.	Focal necroses.		
2	1,670	2	1 (59–F)	2	0	" "	" "	Infiltration of leukocytes.	
3	1,900	2	1 (59–F)	7	0	Endocarditis, acute. Pericarditis, acute.	0	Parenchymatous changes.	
4	2,230	3	1 (59–F)	7	0	Focal necrosis and infiltration.	0	Infiltration of small round cells.	Streptococcus recovered from heart's blood at autopsy.
5	1,850	3	1 (59–F)	7	0	Focal necrosis.	0	Severe parenchymatous nephritis, focal necroses.	"
6	1,715	9	4 (A–30)	26	0	0	Infiltration of leukocytes in lobules.		
7	1,620	0	3 (A–30)	18	Acute. Left wrist.	Acute pericarditis.	Infiltration of lymphoid cells.	Infiltration of lymphoid cells.	Died of pneumonia.
8	1,720	7	4 (59–F)	26	Subacute. Left shoulder.	0	0	Collection of lymphoid cells in cortex.	

9	1,800	0	3 (59–F)	12	Acute. Both ankles, left wrist, right shoulder.	0	Diffuse infiltration of round cells.	Collection of lymphoid cells in cortex.	Died of pneumonia. Streptococcus from joints.
10	670	6	1 (A–30)	8	Acute. Right knee.	0	Marked cloudy swelling.	Collection of lymphoid cells.	
11	554	0	1 (A–30)	1	0	0	0	0	Culture from heart's blood sterile.
12	692	18	4 (59–F)	70	Subacute. Right elbow, left shoulder.	Focal necrosis and infiltration. Repair.	Focal necroses, early cirrhosis, endocarditis.	Collection of lymphoid cells.	
13	490	1	1 (59–F)	1	0	0	0	0	Died.
14	634	9	2 (A–49)	19	Acute. Both knees and shoulders.	0	0	Collection of lymphoid cells.	
15	624	6	2 (A–49)	17	Acute. Both shoulders, left wrist.	A few foci of round cell infiltration.	Focal necroses.	"	
16	635	1	1 (59–F)	1	0	Foci of round cell infiltration.	0	0	Died.
17	600	3	1 (59–F)	8					Incomplete autopsy.
18	425	5	1 (A–32)	6	0	Focal necrosis and round cell infiltration.	Small adenomata of ducts.	0	
19	779	6	1 (59–F)	19					Incomplete autopsy.
20	500	6	1 (A–32)	7	0	0	Coccidia.	0	

TABLE I—*Continued.*

No. of rabbit.	Weight.	No. of tryan blue injections.	No. of streptococcus injections.	Duration of life after 1st injection of streptococci.	Arthritis.	Lesions in heart.	Lesions in liver.	Lesions in kidneys.	Remarks.
	gm.			*days*					
21	682	2	1 (59–F)	1					Incomplete autopsy.
22	1,610	14	20 (59–F)	225	Chronic. Both shoulders, left wrist.	Foci of round cell infiltration.		Infiltration of lymphoid cells.	
23	1,480	14	13 (A–26)	180	Chronic. Both shoulders.	0		0	
24	1,800	14	17 (A–32)	180	Chronic. Right shoulder.	0		0	
25	1,895	4	8 (59–F)	40	Subacute. Both shoulders.	Acute endocarditis and pericarditis. Foci of round cell infiltration. Thrombosis of vessels.		0	
26	1,820	14	8 (59–F)	50	Subacute. Both shoulders.	A few foci of cellular infiltration.		Parenchymatous changes.	
27	1,880	14	8 (59–F)	140	0	0	Moderate cirrhosis.	0	
28	1,760	14	8 (59–F)	50	Subacute. Both shoulders.	Foci of necrosis and cellular infiltration.		0	
29	1,830	14	5 (59–F)	50	0	0		0	
30	2,050	9	2 (A–49)	14	0	0	Central necroses.	0	
31	2,490	18	3 (A–49)	28	Acute. Right elbow.	Foci of cellular infiltration. Repair.	Coccidia.	Collection of lymphoid cells.	Streptococcus recovered from joints.

32	2,200	8	1 (A–49)	10	Acute. Right elbow, left knee.	0	0	Slight sclerosis, infiltration of lymphoid cells.	Streptococcus recovered from joints.
33	2,295	18	3 (A–49)	28	Acute. Both elbows and knees, both wrists and left ankle.	0	0	Collection of lymphoid cells.	"
34	2,640	14	2 (A–49)	21	Acute. Right knee. Synovitis, right leg.	Foci of necrosis and cellular infiltration.	0	0	"
35	1,890	19	3 (A–49)	26	Acute. Left shoulder.	Foci of cellular infiltration.	0	Marked parenchymatous nephritis.	Rabbit received very large doses of streptococci (100–200 cc.) (broth culture).
36	1,700	17	2 (38-D)	12	Acute. Synovitis, both heels.	Large foci of necrosis, cellular infiltration, and repair.	0	Parenchymatous nephritis, infiltration of lymphoid cells.	Rabbit received very large doses of streptococci.
37	1,760	6	1 (38-D)	1	0	0	Central necroses.	0	"
38	1,810	6	1 (38-D)	1	0				
39	1.580	16	0		0	None. Many macrophages.	Many macrophages.	0	Control.
40	1,680	23	0		0	" " "	" "	0	"
41	1,770	23	0		0	" " "	" "	0	"

no streptococci. The rabbits were usually of medium size, varying in weight from 425 to 2,500 gm. For one series, an attempt was made to use five young rabbits, but their veins were not well adapted to the trypan blue injections.

Six different strains of streptococci were employed for the experiments. They were obtained from blood culture from the following sources:

1. Rheumatic fever (59–F).
2. Rheumatic fever (A–49).
3. Rheumatic fever (38–D).
4. Infectious endocarditis (subacute) (A–30).
5. Infectious endocarditis (subacute) (A–32).
6. Infectious endocarditis (subacute) (A–26).

The six strains all belonged to the non-hemolytic group of streptococci. Four were green producers, while two, A–26 and A–32, produced no change whatever in blood.

The number of streptococcus injections varied from one to four and were separated by 5 to 10 day intervals. The average number of injections was two. This excludes the eight immunized animals (Nos. 22–29) which first received several injections of killed streptococci and then a series of injections (five to twenty) of living streptococci.[1] The dosage of streptococcus culture used in my experiments has varied considerably. In the earlier work I used 5 to 20 cc. of a 24 hour broth culture. In the later experiments much larger doses were given (three to five rabbit blood agar slants), and in the last series 100 to 150 cc. of a broth culture were injected at one time.

RESULTS.

Joint Lesions.

In this investigation I have given considerable study to the pathology of the joints, for in reviewing the literature it was noticeable that very little attention has been paid to the pathological anatomy of arthritis, either in the human being or experimental animal. Poyn-

[1] The privilege of studying the lesions in the immunized rabbits was granted to me by Drs. Swift and Kinsella who were employing them for the study of another problem.

ton and Paine (16), Cole (17), and Jackson (8) have described the gross, and to some extent the microscopic appearance, but the cellular changes have not been carefully studied. In every case of arthritis in the present series, sections have been taken through the joints involved and studied microscopically. The sections were all decalcified in Müller's fluid, which did not injure the vital stain to any great extent. The sections were cut by the paraffin method and stained (1) with hematoxylin and eosin, (2) with aqueous cochineal (to bring out vitally stained cells), and (3) by the Gram-Weigert method for bacteria. The Unna-Pappenheim stain could not be used on account of the formalin fixation. By referring to the table it will be seen that of the thirty-eight rabbits injected with streptococci, nineteen, or 50 per cent developed arthritis; in seven the disease was monarticular, and in twelve polyarticular. The shoulder was the joint most frequently involved; the right nine times, the left ten. The other large joints were affected with about equal frequency. In many cases it was difficult to determine the exact duration of the arthritis, for the reason that the rabbit showed no symptoms. Many infected joints were not discovered until the autopsy was performed.

The rabbits with arthritis lived from 8 to 225 days after receiving their first injection of living streptococci. Four animals lived less than 2 weeks; eight of the nineteen rabbits lived from 2 to 4 weeks after their first injection. The remaining seven lived from 6 to 32 weeks.

The four rabbits that lived or were killed in less than 2 weeks after receiving streptococci (Nos. 9, 10, 32, and 36) had arthritis in a very acute form. Two of these rabbits received only one injection each of streptococci; another received two, and the last three injections. Faber (18) found that it usually took more than one injection of streptococci to produce arthritis. As a rule, this is true, but not invariably, as our results show. In the gross these joints were moderately swollen and contained bluish viscid mucoid material, films of which showed many pus cells filled with coarse blue granules of trypan blue. In two of the cases streptococci were recovered in cultures from the joint exudate. Microscopical sections through the infected joints show extensive infiltration of the synovial membrane with leukocytes, chiefly of the polynuclear type. In addition there

are present a few large endothelioid cells (macrophages) which contain blue granules and are similar in every respect to the large cells found in the joint exudate. The membrane is thickened from edema and cellular infiltration. The synovial membrane is covered with an exudate of leukocytes and a few endothelioid cells. The cartilage is intact and shows no infiltration. The marrow shows a slight hyperplasia. The capsule is little if any changed.

In the second group of cases that lived for 2 to 4 weeks after the first injection of streptococci (Nos. 7, 8, 14, 15, 31, 33, 34, and 35), the gross appearance of the infected joints differed in no way from those of the first group.

Microscopically, however, there are some differences. In these rabbits the infection is obviously of longer duration. In some places the cartilage is superficially necrotic and the destroyed portion is being replaced by connective tissue. The bone marrow shows considerable hyperplasia. The synovial membrane is thickened and densely infiltrated with leukocytes and the same large endothelioid cells filled with blue granules (Fig. 1). Lymphoid and plasma cells are more numerous in the infiltration and exudate than in the first group.

There are signs of beginning organization in the exudate and it is interesting in this connection to study the part of the large endothelioid or macrophage cells. On the surface they appear as large round, or oval cells packed with coarse blue granules. Deeper down in the exudate near the synovial membrane these cells have apparently become pulled out into fusiform and stellate shapes, appear smaller, and contain fewer and finer granules. The impression is obtained that the macrophages, having accomplished their first duty as phagocytic cells, have become gradually transformed after this function is completed into fibroblasts to assist in the process of repair. Eventually they become adult connective tissue cells and lose their phagocytic power entirely.

In the third group of cases (Nos. 12, 22, 23, 24, 25, 26, and 28) many of the infected joints began to show chronic changes, such as great thickening of the capsule with considerable limitation of motion; the bone was very friable and crumbled easily when sections were sawed for microscopical examination. There was no bony ankylosis, however, in any of the cases.

Microscopical sections through these joints show extensive necrosis of the cartilage which sometimes extends down into the bone. There is marked hyperplasia of the marrow which has replaced the cancellous bone to a great extent. The synovial membrane is thickened and the deeper part composed of newly formed connective tissue; the superficial part is very cellular. The cells are leukocytes, mostly lymphoid and plasma cells, though there are a moderate number of polynuclear cells also present and large numbers of macrophages which appear to be undergoing the transition into fibroblasts, as just described. No bony changes were observed, even in the more chronic cases, but there were hyperplasia of the bone marrow and occasional thrombi or foci of granulation tissue in the marrow.

It will be seen from these descriptions that the changes which take place in experimental arthritis represent a gradual transition from a stage of acute inflammation in which there is little more than an infiltration of leukocytes into the synovial membrane and an outpouring of mucoid material, leukocytes, and macrophages into the synovial fluids to a stage of subacute or chronic inflammation in which the following changes occur. The synovial membrane and the cartilage undergo more or less necrosis. The cellular exudate in the synovial membrane consists chiefly of lymphoid cells and macrophages. There is marked hyperplasia of the bone marrow with more or less absorption of the cancellous bone. It is possible that if some of these rabbits had lived long enough, new formation of bone and possibly ankylosis would have occurred, as described by Poynton and Paine. The capsule of the joint thickens as a result of the long continued inflammation. The changes resemble those observed in cases of acute infectious arthritis in man which become subacute and finally pass into the chronic stage of arthritis. From the standpoint of the pathology and histology of experimental arthritis, there is little to differentiate it from human arthritis of the infectious or metastatic type. It has been observed by previous investigators of experimental streptococcus arthritis that complete recovery of the joint may take place as in human rheumatism. Unfortunately, I have not had an opportunity to study any joints of this type.

Cardiac Lesions.

The hearts were studied in considerable detail and it was chiefly in the hope of throwing some light on the foci found in the heart in experimental streptococcus arthritis that the rabbits were vitally stained. Almost all the sections studied were from the left ventricle, and particular care was taken to include the papillary muscles in the sections.

Of the thirty-eight rabbits injected with the streptococci, eighteen showed pathological changes in the heart or pericardium; eleven of the eighteen belonged also to the arthritis series. In three rabbits complete autopsies were not performed. Rabbit 3 showed an endocarditis and pericarditis. In Rabbit 25 a vegetative endocarditis was found associated with pleurisy and pneumonia. Rabbit 7 had a pericarditis. In the remaining fifteen the lesions were in the myocardium. These lesions were usually not visible with the naked eye on account of the trypan blue stain. The earliest focal lesions observed have been small areas of necrosis in the muscle fibrillæ (Fig. 2). At first these consist only of necrotic fragments of muscle with here and there a surviving nucleus. At this stage no infiltration of leukocytes or wandering cells has occurred. These foci are usually small, but occasionally one sees necrotic areas of considerable extent situated in the muscle, never in the supporting stroma, and sharply defined from the surrounding healthy parenchyma. The line of demarcation from healthy tissue is emphasized by the vital staining of the necrotic foci, which take a light blue color due to the deposition in them of very fine blue granules of trypan blue. A later stage of such focal necroses is characterized by the presence of leukocytes, usually of the lymphoid type, and macrophages filled with blue granules (Fig. 3). Focal necroses were observed in nine cases. In three of these (Nos. 1, 2, and 5) the focal necroses were the only lesions present. The other six (Nos. 4, 12, 18, 28, 34, and 36) showed in addition to necrosis more or less extensive cellular infiltration. In seven other cases, foci of infiltration, chiefly by lymphoid cells, were present with little or no necrosis (Fig. 4).

The foci of cellular infiltration were of particular interest, especially as regards their resemblance to Aschoff's bodies. The foci

with necrotic centers surrounded by leukocytes and macrophages (Fig. 3) bore the nearest resemblance to the submiliary nodules of rheumatic fever, but even in these the resemblance was not close. In the first place, the location of the lesions in the rabbit heart is different from that in the human heart. In the rabbit I have always found the necrotic foci in the heart muscle proper and not related in any way to the blood vessels. Sometimes smaller collections of lymphoid and macrophage cells are found in the stroma, but this is the exception rather than the rule. The submiliary nodule is always found in the supporting connective tissue, usually about a blood vessel. In the rabbit's heart the necrosis is definite; in the human heart it is slight and of the hyaline type. There is a difference, too, in the cellular exudate about the necrotic foci. In the rabbit the cells are chiefly lymphoid and plasma cells and large macrophages. In the human lesion there are, in addition to these types, a considerable number of giant cells which resemble those seen in Hodgkin's disease. Furthermore, the radial arrangement of the cells in a submiliary nodule is characteristic, whereas in the experimental nodule the cells are arranged in a purely haphazard manner.

In Rabbit 36 I succeeded in demonstrating streptococci in one of the myocardial lesions. This rabbit, however, had received such large doses of streptococci that perhaps too much significance should not be attached to this point. Thalhimer and Rothschild were unable to demonstrate bacteria in the myocardial foci which they studied, and I have not found them in any other of my own cases.

In a number of cases, especially Nos. 12, 31, and 36, there were signs of repair (Fig. 5), and here, as in the joints, the macrophages seemed to play an important part. They were present in large numbers and were apparently being converted into fibroblasts.

It may be said, then, that while the myocardial lesions in experimental streptococcus arthritis follow in a general way the course of those occurring in the rheumatic human heart,—that is, necrosis and infiltration and finally proliferation with the formation of scars,—the histological appearance of the two lesions when carefully studied, is different.

Endocarditis and Pericarditis.

Sections through the vegetations on the heart valves in Rabbits 3 and 25 showed a fibrinous network, partially organized, and infiltrated with leukocytes and a moderate number of macrophages. Sections through the pericardial exudates presented similar pictures (Rabbits 3, 7, and 25).

Lesions in the Kidneys.

In the study of the rabbit's kidneys the investigator is always handicapped by the frequency of pathological changes in supposedly normal kidneys. Christian, Smith, and Walker (19), Ophüls (20), Longcope (21) and others have found round cell infiltration and patches of sclerosis in 22 to 33 per cent of kidneys of normal rabbits.

In the present series of thirty-seven cases in which the kidneys were examined microscopically, the kidneys of the three controls, from rabbits that received trypan blue but no streptococci, were normal; and of the remaining thirty-four from rabbits that had received injections of streptococci, eighteen, or 52 per cent, showed definite pathological changes. Of these, fourteen were associated with arthritis. As in all there were nineteen cases of arthritis, it follows that fourteen out of the nineteen, or 74 per cent, were associated with renal changes. In nine cases the lesion consisted of collections of lymphoid cells in the cortex with more or less patchy sclerosis. In four cases there was a well developed parenchymatous nephritis, with extensive destruction of the tubular epithelium of the convoluted tubules. In one case both parenchymatous and interstitial changes were noted (No. 36).

The glomerular changes in streptococcus infections have been studied by Baehr (22), Löhlein (23), and others. Baehr has described characteristic glomerular lesions in infectious endocarditis due to the lodgment of bacterial emboli in the glomerular capillaries.

Lesions in the Liver.

In six cases the liver showed focal necroses, which were usually about the central veins and were themselves in most cases surrounded by a zone of leukocytes. In only two of the six cases was

there an associated arthritis. In two other cases there was a well marked cirrhosis (Nos. 12 and 27). A slight degree of round cell infiltration and cirrhosis was present in some of the other cases, but the change was not sufficiently marked to be significant.

Lesions in Other Organs.

The spleen and lymph glands were enlarged in all the rabbits. Microscopical sections showed hyperplasia of the lymphoid tissue and large numbers of macrophages. These changes were apparently due to the vital stain as they were also observed in the control rabbits. In Rabbit 9 there were infarcts in the spleen.

The lungs showed nothing of interest except in two cases. Rabbits 7 and 9 both died of pneumonia. Microscopical sections showed the usual exudate in the pulmonary alveoli.

DISCUSSION.

The injection of one or more doses of *Streptococcus viridans* into the vein of a rabbit produces in many cases an acute arthritis which bears some resemblance to the arthritis of rheumatic fever, but is perhaps more nearly analogous to the so called acute or subacute infectious arthritis so frequently associated with focal infections in the teeth, tonsils, or other parts of the body. Microscopically, joint sections from vitally stained animals show in addition to numerous leukocytes, a considerable number of large endothelioid cells or macrophages, which take the vital stain. These cells migrate from the tissue into the joint exudate and could not be seen to develop from the endothelial lining of the joint as commonly supposed. As the infection becomes older these macrophages become more and more numerous and, gradually losing their phagocytic power, appear to develop into fibroblastic cells. In the chronic stage the infected joints show ulceration of the cartilage and synovial membrane, with hyperplasia of the bone marrow and fibrous thickening of the capsule.

The most interesting changes in the heart are the myocardial lesions, which may be briefly described as small foci of necrosis infiltrated and surrounded in most cases by lymphoid cells and macrophages. The location of these lesions in the heart muscle proper, the

arrangement of the cells, and the absence of giant cells distinguish them sharply from Aschoff bodies. In a small percentage of cases bacterial endocarditis or pericarditis occurs.

The lesions produced in other organs are not especially characteristic. Focal necroses are frequently found in the liver, and the kidneys often show foci of round cell infiltration. In the more chronic cases there may be some production of fibrous tissue.

The large endothelioid cells in the heart muscle appear to develop from the vascular endothelium. In this respect my observations agree with those of Evans. In many sections the endothelial sprouts can be seen already developing phagocytic power, as shown by the presence in them of the trypan blue granules.

In the joint exudates the origin of the macrophages is not so clear. One thing is obvious in all the organs; namely, that the injection of trypan blue stimulates an increased production of macrophages.

In the three control rabbits that received no streptococci but were injected with trypan blue alone, the increased number of macrophages was noticeable in all the organs, but strikingly so in the liver and heart muscle.

CONCLUSIONS.

1. Repeated injections into rabbits of non-hemolytic streptococci isolated from human cases of infectious endocarditis or rheumatic fever will produce an acute arthritis in the rabbit similar in most respects to the arthritis of acute rheumatism.

2. Microscopical sections of the joints show a gradual transition from an acute exudative inflammation to advanced organization.

3. Endocarditis and pericarditis occur in a small percentage of cases, and focal lesions in the myocardium consisting of necrosis and the infiltration of cells are frequent. These focal lesions differ considerably from Aschoff's submiliary nodules.

4. Lesions in the kidneys and liver occur but are not characteristic.

5. By means of the vital stain it has been shown that the large endothelioid cells which play a prominent part in the joint and myocardial lesions belong to the group of so called macrophages or wandering cells and probably develop from the vascular endothelium.

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EXPLANATION OF PLATES.

PLATE 49.

FIG. 1. Rabbit 8. Section through capsule of left shoulder. Subacute arthritis. The membrane, which is considerably thickened, shows internally a layer of necrotic material beneath which the tissue is densely infiltrated with macrophages and leukocytes. Leitz obj. 3, oc. 4.

FIG. 2. Rabbit 5. Focal necrosis in heart muscle. No cellular infiltration has occurred. Leitz obj. 5, oc. 4.

PLATE 50.

FIG. 3. Rabbit 36. Focus in heart muscle, showing necrotic center, surrounded by macrophages and lymphocytes. Vitally stained cells contain blue granules. Counter-stained with aqueous cochineal. Zeiss obj. 5, oc. 4.

PLATE 51.

FIG. 4. Rabbit 12. Focus in heart muscle, showing infiltration of lymphoid cells. Slight necrosis. Leitz obj. 5, oc. 3.

FIG. 5. Rabbit 31. Focus in myocardium showing repair. A few macrophages and lymphoid cells, but connective tissue cells predominate. Leitz obj. 7, oc. 3.

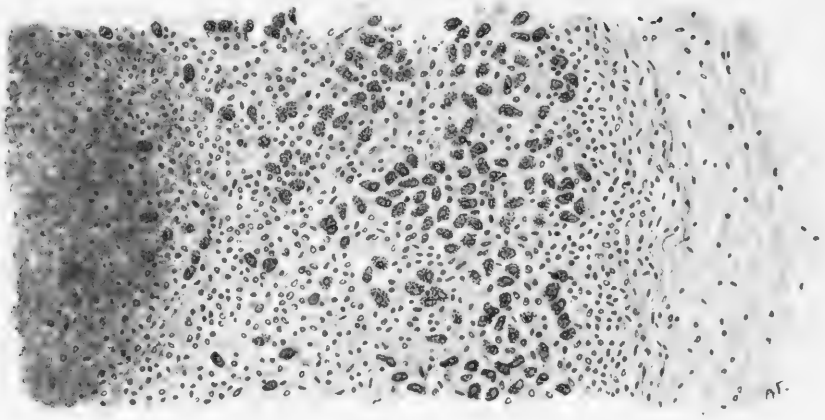

FIG. 1.

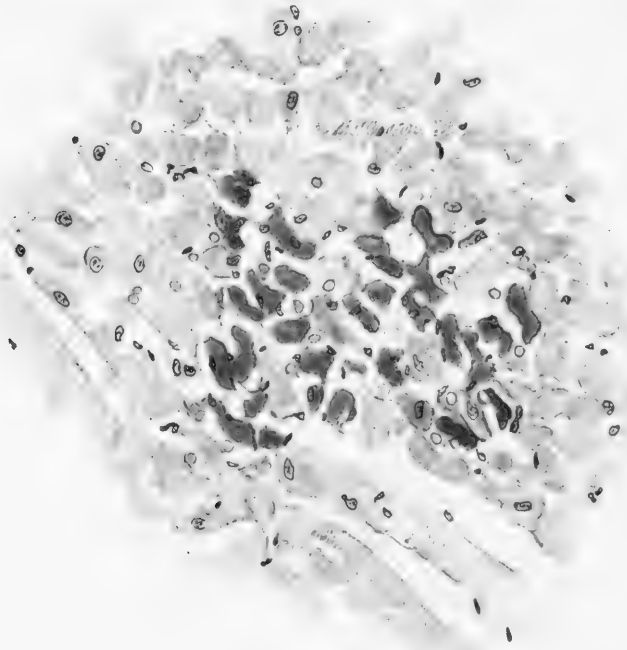

FIG. 2.

(Cecil: Non-Hemolytic Streptococcus Lesions.)

FIG. 3.

(Cecil: Non-Hemolytic Streptococcus Lesions.)

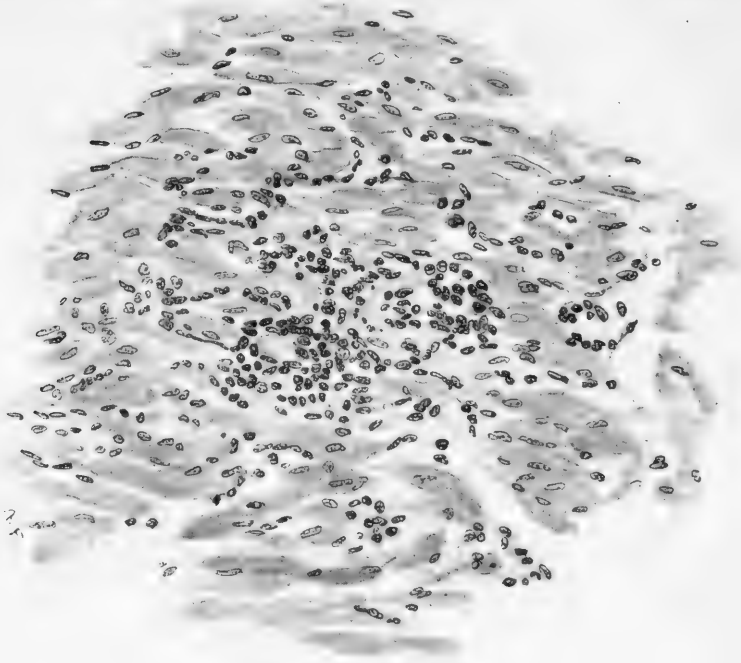

FIG. 4.

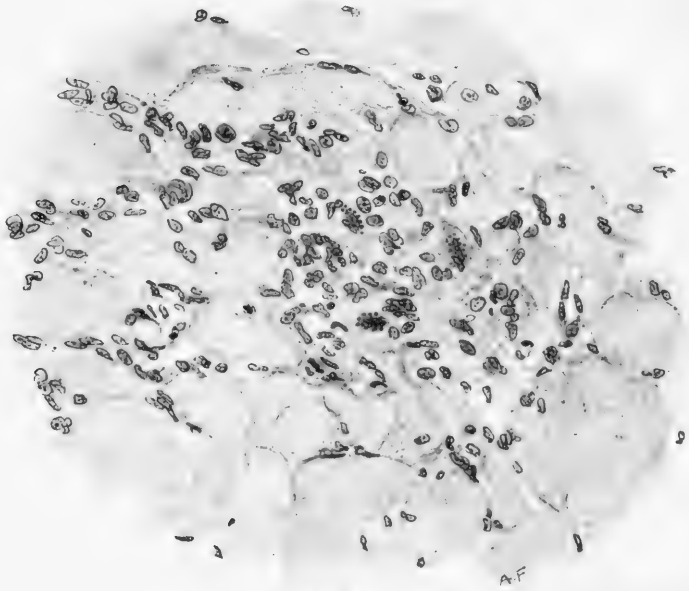

FIG. 5

(Cecil: Non-Hemolytic Streptococcus Lesions.)

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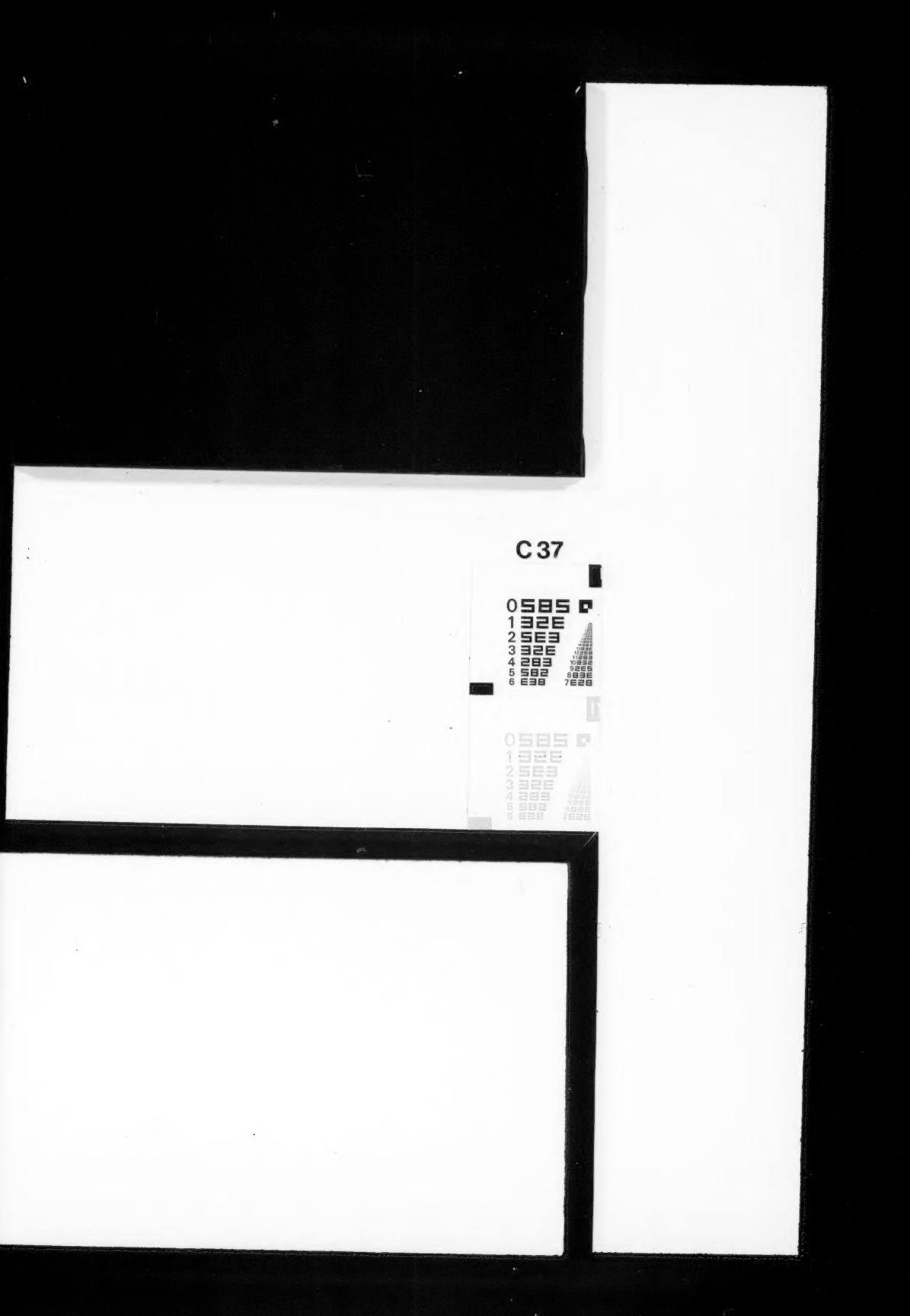
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